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*METABOLIC EXCHANGE OF CARBON DIOXIDE WITH CARBOXYLS AND OXIDATIVE SYNTHESIS OF C_4 DICARBOXYLIC ACIDS**

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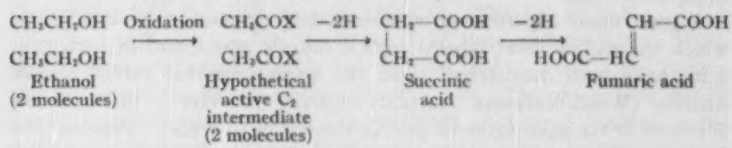
Communicated by S. C. Lind, February 11, 1950

Recently[1] we advanced evidence that fumaric acid synthesis from ethanol in strain No. 45 of the mold *Rhizopus nigricans* takes place by direct condensation of two C_2 moieties according to this scheme:



The main findings were: *Specific* activity of the fumaric acid methine groups was exactly equal to that of the ethanol methyl groups; one mole of fumaric acid formed from methyl C^{14}-labeled ethanol contained *more* than the total radioactivity of the two moles of substrate ethanol; the carboxyl groups of fumarate contained all the radioactivity in excess of that in the two moles of ethanol utilized in the synthesis; some of the substrate ethanol was oxidized to CO_2 and, therefore, contained substantial radioactivity which accumulated in the closed system.

A mole of fumaric acid formed from carbinol C^{14}-labeled ethanol contained less radioactivity than the two moles of substrate ethanol; there was no radioactivity in the methine groups of the fumaric acid; the carboxyls contained all the radioactivity; radioactive CO_2 was generated by complete oxidation of a portion of the substrate alcohol, and it accumulated in the closed system.

The foregoing was interpreted to mean that the fumaric acid produced at any instant from methyl-labeled ethanol would have zero radioactivity in the carboxyl groups. However, this fumaric acid is in metabolic

equilibrium with malic and oxalacetic acids which are known to undergo reversible enzymatic decarboxylation by specific enzymes present in this organism.[2-4] Respiratory carbon dioxide liberated by decarboxylation of the initially unlabeled carboxyl groups mixes with radioactive carbon dioxide in the vessel atmosphere so that during the reversible decarboxylation, the C_4 acid now acquires radioactivity in the carboxyls.

The carboxyl groups of the fumaric acid formed at any instant from carbinol-labeled ethanol would contain radioactivity equal to the carbinol group of the ethanol. However, the carbon dioxide in the atmosphere here originates from both carbons in the ethanol, and also from endogenous respiration of the mold, hence has a specific radioactivity much less than the carboxyls of the newly formed fumarate molecules. Consequently, during reversible decarboxylation the carboxyls become diluted with carbon dioxide of lower specific radioactivity, thereby accounting for the presence of less radioactivity in the fumarate than was contained in the two alcohol molecules generating it. That nonradioactive carboxylic acids can acquire radioactivity in the carboxyl groups from labeled radioactive carbon dioxide during the action of the corresponding decarboxylases has been convincingly demonstrated in the instances of oxalacetic acid and α-ketoglutaric acid.[4, 5]

A great many experiments on intermediary respiratory metabolism in which the carbon from labeled carbon dioxide was found in carboxylic acids have been interpreted[6-11] on the assumption that carbon dioxide fixation (Wood-Werkman reaction) actually provides a mode of net synthesis of the acids (over-all gain in amount of C_4 acids). Fumaric acid synthesized by *R. nigricans* from inactive ethanol in the presence of $C^{14}O_2$ contains C^{14} in the carboxyl groups,[1] a finding seemingly in harmony with the numerous above-mentioned experiments of this type which have been interpreted as a *net* synthesis utilizing carbon dioxide. However, our previous experiments with labeled ethanol make it reasonably certain that the fumarate originated by a mechanism independent of carbon dioxide fixation. In consequence of the universal character of reversibility of enzyme action, the $C^{14}O_2$-inactive ethanol experiment would seem to represent metabolic exchange between carboxyl and respiratory carbon dioxide and thus support our interpretations relative to the distinctive changes observed in the carboxyls of fumaric acid produced from *labeled* ethanol.

Although, as discussed below, a different mechanism could account for some of the observed results, the possible broader significance for tracer studies implied by the discovery of this carbon dioxide metabolic exchange with preformed C_4 acids makes additional study of the problem desirable.

Methods.—General methods and materials were precisely as described previously[1] except that the experimental vessels were 400-ml. beakers

instead of 250-ml. Erlenmeyer flasks in order to facilitate physical gas exchange.

Experiments.—Carbon dioxide being a reactant in the system, C_4 acid $\overset{\text{decarboxylase}}{\rightleftharpoons}$ C_3 acid + CO_2, its partial pressure would be expected to influence the carboxyl labeling, and these experiments center on this point. In our previous experiments the respiratory carbon dioxide was allowed to accumulate in the closed vessels (10-liter desiccators) for the duration of the experiment; this reservoir of (radioactive) carbon dioxide was assumed to be that utilized in the exchange reactions. Starting with normal air atmosphere and 25 ml. of 2% methyl-labeled ethanol, the carbon dioxide concentration in the gas phase reached 1 to 1.5% in 4 to 5 days under these conditions.

In this work a similar normal air treatment was set up as the reference point. Three other methyl-labeled ethanol treatments using portions of the same batch and amount of mycelium were run at the same time. They were identical in every respect except for differences in carbon dioxide tensions.

Treatment: (1) Ordinary air atmosphere; respiratory CO_2 allowed to accumulate. (2) Strong NaOH to absorb respiratory CO_2 keeping the CO_2 content of the gas phase nil. (3) 5% CO_2 containing 1.47 atom per cent $C^{13}O_2$; 20% O_2; 75% N_2. (4) 15% CO_2; 20% O_2; 65% N_2.

In treatment (2) the NaOH (30 ml. 6 *N*) was contained in a 50-ml. beaker suspended rigidly inside the 400-ml. beaker holding the culture and just above the surface of the culture liquid. The object here was to insure immediate absorption of CO_2 produced as close as possible to the surface of the liquid. Fluted filter paper in the alkali beaker increased the absorptive surface. The alkali beaker was fixed in position by fusing it to the larger beaker with thin glass rods. Treatment (3) was designed to increase the rate of exchange between carbon dioxide and carboxyl as a result of increased tension of carbon dioxide (5%). An additional quantitative check on entrance of extracellular carbon dioxide into carboxyl groups was obtained by adding $C^{13}O_2$ to the gas phase. A carbon dioxide concentration of 5% also minimizes concentration changes due to evolution of respiratory carbon dioxide, which otherwise would complicate the quantitative aspects of $C^{13}O_2$ utilization. The carbon dioxide content of the gas phase was considered to have remained constant in this experiment (less than 20% increase). Another purpose of treatment (3) was to have the $C^{14}O_2$ of respiration substantially diluted with the 5% nonradioactive carbon dioxide present in the atmosphere, resulting in a lowered specific radioactivity of the extracellular carbon dioxide available for exchange with the carboxyls of the C_4 dicarboxylic acids. Theoretically, this would reduce appreciably the specific activity of the carboxyls as compared to treatment (1).

Treatment (4) with a 15% carbon dioxide atmosphere was designed to obtain the maximum dilution of radioactive respiratory carbon dioxide, thereby achieving the lowest possible radioactivity in the carboxyls. Preliminary experiments indicated fumaric acid formation from ethanol is completely inhibited when the carbon dioxide tension exceeds 25% and about one-half normal yields are obtained in 15% carbon dioxide. Consequently, treatment (4) was set up in duplicate desiccators to obtain sufficient fumaric acid for isolation and degradation.

The desiccators containing the beakers fixed in position were shaken on a machine at 28° C. for 4 days. Analyses on the clear filtrates gave the following data:

	FINAL pH	TOTAL FUMARIC ACID FORMED, MG.
Treatment (1)	2.9	69
(2)	2.9	43
(3)	2.9	54
(4)	3.25	21.6
	3.15	20.1
		41.7

Crystalline fumaric acid was recovered from treatments (3) and (4) essentially by the procedure described previously;[1] yields were 39 and 18 mg., respectively. The fumaric acid in treatments (1) and (2) was obtained by continuous ether extraction. The fumaric acid was dissolved in water after evaporation of the ether; titrimetric estimation of the fumaric acid was made on these aqueous solutions. Other acids are not present in amounts exceeding 1% of the fumaric acid. These two solutions and the two crystalline fumaric acid preparations were then examined for specific radioactivity of the carboxyl groups as the criterion of metabolic exchange of carbon dioxide with carboxyls.

The following procedure was developed for the advantage it has of being specific for carboxyl groups of fumaric acid (and acids convertible to fumaric acid, especially succinic acid). Approximately 0.33 mM. fumaric acid in 40-ml. $M/10$ phosphate buffer at pH 7.4 was treated with 10 g. of enzyme solids of a fumarase preparation from beef heart. This preparation is identical with the succinoxidase preparation ordinarily used for manometric estimation of succinic acid.[12] During shaking at 37° C. for 1 hour, the bulk of the fumarate is converted to *l*-malic acid. In a test run in which residual fumaric acid was determined by hydrogenation in the presence of palladium, the final equilibrium mixture contained fumarate and malate in the ratio of 1 to 4.7. The suspension of enzyme solids was centrifuged, the cloudy supernatant acidified with H_2SO_4 to ~pH 1 and the organic acids extracted with ether for 3 days in a Kutscher-Steudel apparatus. After evaporation of the ether, the acids were taken up in

water and adjusted to pH 5.5. A blank consisting of enzyme acting on buffer alone was carried along exactly as the test run.

The malic acid was then decarboxylated by a suspension of *Lactobacillus casei* yielding lactic acid and carbon dioxide quantitatively.[18] Starting with known amounts of *l*-malate, we have confirmed the stoichiometry of this decarboxylation, and have demonstrated its specificity particularly with respect to related acids potentially present as impurities in biochemical work of this kind. Under the same conditions that one mole of carbon dioxide is liberated from malate, the bacteria were completely inert against fumarate, citrate, succinate and α-ketoglutarate, singly and in combination.

Cells from 200 ml. of malate medium (glucose 1%, *l*-malate 0.25%, yeast extract 0.1%, phosphate buffer pH 6.6, 0.5%) were centrifuged after 40 hours' incubation at 28° C., washed twice in $M/10$ phosphate buffer at pH 5.5 and suspended in 3 ml. of the same phosphate buffer. This thick suspension was placed in one side arm of a large (160-ml.) Warburg vessel and 1.0 ml. of 10 N H_2SO_4 in the second side arm. The solution containing malic acid at pH 5.5 ($\sim$40 ml.) was in the main chamber. After temperature equilibration, the bacteria and the malate solution were mixed; carbon dioxide evolution is virtually complete in about 1 hour, after which bound CO_2 is liberated by the H_2SO_4. Later it was found that the decarboxylation is fully effective at pH 4, obviating the need for the acid dump. In the test run cited above, where malate was generated from fumarate by fumarase, recovery of C_4 acids was 100%. Fumaric acid taken = 0.40 mM.; fumaric acid left, determined by hydrogenation = 0.07 mM.; malic acid, by difference = 0.33 mM.; malic acid determined by bacterial decarboxylation = 0.33 mM.

The ether extraction is an essential step in this determination. The cloudy supernatant from the fumarase treatment retains considerable amounts of apoenzymes of oxidation systems and these are activated by coenzymes leaching from the bacterial cells; complete oxidation of the residual fumarate to carbon dioxide follows, yielding fallacious results. Curiously, malate itself is not oxidized under these conditions notwithstanding the fact that fumarate oxidation might be expected to proceed via malate. Carbon dioxide from a bacterial blank on buffer alone generally is a negligible proportion of the test runs.

The carbon dioxide from malate decarboxylation is collected in an evacuated 500-ml. bulb containing CO_2-free alkali. The bulb is attached to one venting plug of the Warburg vessel, which is flushed by carbon dioxide-free air down through the vessel from the other venting plug. The carbon dioxide is then precipitated as $BaCO_3$, digested on a water-bath, filtered onto tared porcelain counting disks, dried, weighed and taken for radioactivity measurements. This procedure recovers, as $BaCO_3$,

in excess of 95% of the manometrically determined CO_2. All radioactivity results are expressed as specific activity (counts/sec./mg. $BaCO_3$). After radioactivity measurements, the C^{13} in the $BaCO_3$ from treatment (3) was analyzed in a mass spectrometer.

Results.—Because of the limited amounts of fumaric acid obtained in each of the four treatments, specific activity measurements on an aliquot of each fumarate were not attempted. Also, they are unnecessary for this work in which the specific activity of only the carboxyl groups is of importance. However, we have repeatedly shown that mixing of methine and carboxyl groups of fumaric acid (produced from methyl and carbinol of ethanol) does not occur (see below) and that the specific activity of the methine group is precisely that of the methyl group of the starting ethanol, and two times that of the ethanol itself. This nonmixing is all-important, for, as shown below, it means that carbinol-C cannot become methine-C

TABLE 1

SPECIFIC RADIOACTIVITIES OF CARBOXYL GROUPS OF FUMARATE PRODUCED BY *Rhizopus nigricans* UNDER DIFFERENT CO_2 TENSIONS[a]

TREATMENT	SPECIFIC RADIOACTIVITY OF CARBOXYL GROUPS[b]
(1) Air atmosphere[c]	1.06
(2) No CO_2(NaOH)[d]	1.11
(3) 5% CO_2 containing $C^{13}O_2$[e]	0.91
(4) 15% CO_2	0.80

[a] Cultures consisted of mycelium suspended in 25 ml. 2% methyl C^{14}-labeled ethanol.

[b] Counts/sec./mg. $BaCO_3$. Corrected for carbon dioxide from blank carried from fumarase stage through entire analytical process.

[c] Carbon dioxide allowed to accumulate in desiccator. Concentration of CO_2 in gas phase at end of incubation = 1.5%.

[d] By analysis there was no CO_2 in the atmosphere.

[e] See data on $C^{13}O_2$ below.

of fumarate, but that methyl-C (of ethanol) can become carboxyl-C of fumarate. Table 1 gives the carboxyl data.

Discussion.—The fact that elevated carbon dioxide tensions caused definite dilution of the radioactive carboxyl groups, and in proportion to the carbon dioxide tension, proves that carbon dioxide itself influences in some way the radioactivity of the carboxyls. A not implausible interpretation is that carbon dioxide is metabolically exchangeable with carboxyls, presumably through the action of decarboxylases. Absolute proof is perhaps provided by the fact that extracellular $C^{13}O_2$ is actually found in fumarate carboxyls. Fumarate itself having been established[1] to arise by a $2C_3$ condensation would appear to rule out the entrance of $C^{13}O_2$ via the Wood-Werkman reaction in the conventional sense of *net* synthesis of the C_4 carbon chain from $C_3 + C_1$ moieties.

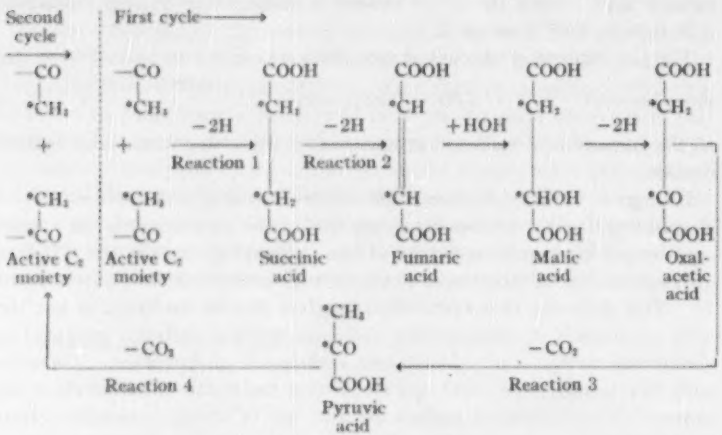
Calculation of Extracellular Carbon Dioxide Exchange with Carboxyls.—

The C^{13} content of the isolated fumaric acid in treatment (3) was found to be 1.13 ± 0.005 atom per cent.[14] 1.13 − 1.09 (C^{13} content of normal CO_2) = 0.04% excess C^{13} in the CO_2 obtained from total combustion of fumaric acid. Since the C^{13} is located in carboxyls only, they contained $2 \times 0.04 = 0.08\%$ excess C^{13}.

The C^{13} content of the initial gas phase was found to be 1.47 ± 0.007 atom per cent. $1.47 - 1.09 = 0.38\%$ excess C^{13}. $\frac{0.08 \times 100}{0.38} = 21\%$ of all the fumaric acid carboxyl groups exchanged with extracellular carbon dioxide.

Changes in Carboxyl Radioactivities in the Absence of Extra-cellular CO_2.—Respiratory Cycles: A most significant fact of this experiment is the virtual equivalence of specific activities of the carboxyl groups in the CO_2-free atmosphere NaOH (treatment 2) vs. air with accumulated CO_2 (treatment 1). This indicates that extracellular carbon dioxide exchange is not the only mechanism of incorporating radioactivity into carboxyl groups, i.e., converting methyl-C of ethanol into carboxyl-C of fumarate. Theoretically two mechanisms could operate to alter the status of carboxyls in the absence of extra-cellular carbon dioxide, and a strong possibility exists that both act simultaneously. They are: (1) exchange with intracellular respiratory carbon dioxide, of which a finite concentration must always be present as long as the fungus cells are metabolically active. In view of the demonstrated participation of extracellular carbon dioxide, it may be reasonably assumed that some internal carbon dioxide also exchanges with carboxyls before it leaves the cell; the quantitative significance of this point is a matter for the future; (2) oxidative conversions of ethanol methyl-C to fumarate carboxyl-C. Rather convincing evidence for a mechanism of this type operating over and above carbon dioxide exchange is provided by the specific activities of the carboxyls in treatments (1) and (2), and the specific activity of the respiratory carbon dioxide in treatment (1), which was determined as 0.85. Thus, if all the carboxyls in treatment (1) had exchanged with carbon dioxide to the extent that they acquired the specific activity of the gas they would still fall short of the observed carboxyl value of 1.06. Thus, a second mechanism independent of carbon dioxide-carboxyl exchange is indicated. The fact that ethanol methyl-C is converted to fumarate carboxyl-C connotes an oxidation, and the assumption that a tricarboxylic acid respiratory cycle takes place in these cells would fit the observed facts. This cycle would, however, differ from the conventional cycle in the respect that the C_4 which condenses with C_2 to generate C_6 arises by $2C_2$ condensation instead of by the generally supposed Wood-Werkman reaction. This may well prove to be one mechanism characteristic of the tricarboxylic acid cycle in carbohydrate metabolism.

Postulation of a dicarboxylic acid respiratory cycle would also fit the observed facts, and this is a distinct and attractive possibility in this instance.



This scheme was formulated for carbohydrate and pyruvate oxidation some 30 years ago, though without proof.[15, 16] During the intervening years good evidence has been gathered for each of the above steps, and Krebs in 1943[10] states that "...the chief weakness of this theory was the complete lack of evidence supporting the assumption of a formation of succinate from acetate." Our data provide, therefore, the means of closing one (if not the last) gap relative to a "dicarboxylic acid respiratory cycle." Four principal steps in this cycle, namely, reactions 1, 2, 3 and 4, have already been demonstrated separately in *R. nigricans* No. 45, and if they operate in sequence, as is likely, a C_4 dicarboxylic acid cycle will in fact have been established. Important to note is the experimental fact that the fumaric acid which accumulates from ethanol in these cultures is rapidly oxidized to completion (i.e., to carbon dioxide) as soon as all the ethanol is consumed.

It can be seen from the above scheme that after the first cycle, the original methyl-labeled C_2 becomes labeled in both carbons. This C_2 labeled in both carbons mixes with more substrate methyl-labeled C_2. The C_4 acids now resulting from a $2C_2$ condensation reaction would be labeled in a manner that matches the experimentally observed labeling in two critical respects, namely, (*a*) the inside carbons (methine-C of fumaric acid) always show the same specific activity as the methyl groups of the starting C_2 moiety regardless of the number of cycles made; and (*b*) the specific activity of the carboxyl groups can never attain the specific activity

of the methine groups so long as substrate ethanol is present. During the relatively long (4 days) duration of the incubation period in this experiment it is probable that the accumulated fumaric acid at any one time represents the differential between the rates of formation and of oxidation of fumarate, both occurring concomitantly.

It is to be emphasized that a tricarboxylic acid cycle would yield results exactly as the dicarboxylic acid cycle and at present it is impossible to discriminate between these two possibilities or to exclude the possibility that both function simultaneously.

Confirmation of the Synthesis of Fumaric Acid by $2C_2$ Condensation.—Because the validity of many of our conclusions depends on the absence of any significant mixing of methine-C (methyl-C of ethanol) and carboxyl-C (carbinol-C of ethanol), we have re-examined this matter more carefully since our first published experiments. Three independent methods of degradation have now been used. One was, in essence, the formic acid procedure described in our previous paper; this determines specific activity of methine carbons of fumaric acid. A specimen of fumaric acid originating from carbinol-labeled ethanol, and having a specific activity of 0.30 was oxidized with acid-permanganate and the formic acid (over-all yield = 89%) in the fourth quarter of a Duclaux distillation taken for radioactivity measurements. The carbon dioxide from oxidation of this formic acid was trapped as $BaCO_3$, which was weighed, and the carbon dioxide liberated for gas ionization measurements with a vibrating reed electrometer. The results were expressed as amperes of ion current per milligram of original $BaCO_3$. Similar measurements were made on the total carbon dioxide from complete oxidation of a portion of the fumaric acid itself. Total fumaric acid = 14.5×10^{-16} amperes net per milligram $BaCO_3$. Formic acid = 0.037×10^{-16} amperes net per milligram $BaCO_3$. The actual amperage here was 1.7 times background. The following expression gives the value for the carboxyl-carbons of the fumaric acid: $14.5 \times 10^{-16} = \frac{2(0.037 \times 10^{-16}) + 2X}{4}$; $X = 28.96 \times 10^{-16}$ amperes per milligram $BaCO_3$ from carboxyl-carbon. Ratio of carboxyl-carbon to methine-carbon $= \frac{28.96 \times 10^{-16}}{0.037 \times 10^{-16}} = \frac{783}{1}$. Thus, the mixing of carboxyl-carbon at most could be 1 part in 783, an insignificant amount. In fact, the steam distillate contained some very small amount of volatile radioactivity which did not follow the formic acid Duclaux curve, and in all probability even the minute activity recorded as formate was due to an impurity and not to formic acid itself.

The second method of degradation consists of measuring the CO_2 from decarboxylation of malate obtained from the above fumaric acid of specific

activity 0.30. The specific activity of this CO_2 was 0.62. Theory = 0.60 (calculated from specific activity of total fumaric acid, which was 0.30, as noted above the methine-C in this fumaric acid contained no radioactivity).

The third method of degradation (details of which will be given in a later publication) employs these principles: fumaric acid $\xrightarrow{\text{fumarase}}$ malic acid $\xrightarrow[L.\ casei]{-CO_2}$ lactic acid $\xrightarrow[H_2SO_4]{KMnO_4}$ acetic acid + CO_2. The acetic acid-carbons represent both methine-carbons of fumarate. The specific activity of methine-carbons of standard fumaric acid sample as determined by the above acetate method was 4.8; that by the formate method on the same sample was 4.8. Thus, three independent methods of degradation agree on the specific activity composition of fumaric acid.

Oxidative Synthesis of C_4 Dicarboxylic Acids and Labeled CO_2 Fixation Experiments.—If our interpretation of the data obtained relative to $2C_2$ condensation is correct in its main conclusion, it means a direct oxidative pathway to the C_4 dicarboxylic acids exists in addition to the Wood-Werkman reaction. Indeed, demonstration in normal tissues of the Wood-Werkman formation of C_4 acids by locating the labeled CO_2 in carboxyls of these acids would not, in view of our experiments, constitute unequivocal proof of *net* synthesis involving carbon dioxide, for metabolic exchange of the carbon dioxide with carboxyls of acids *preformed, and by a route independent of CO_2 fixation* may yield the same results. It would seem that in the absence of evidence excluding the operation of other mechanisms of organic acid synthesis, the incorporation of labeled carbon dioxide into carboxyl groups would have to be interpreted with considerable reserve.

Biosynthesis of Doubly Labeled C_4 Dicarboxylic Acid Tracers.—The results obtained in treatment (3) in the experimental section suggest a useful method of securing labeled C_4 dicarboxylic acids, whose isotopic composition allows the fate of carboxyl-carbons and central-carbons to be followed independently. By using the appropriate methyl-labeled ethanol and the appropriate labeled CO_2, three different kinds of C_4 acids can be produced:

Summary.—Biosynthesis of fumaric acid by a $2C_2$ condensation in the mold *R. nigricans* has been confirmed and extended. It has been demonstrated that CO_2 can undergo metabolic exchange with carboxyls of preformed fumaric acid. According to these experiments demonstration in normal tissues of the Wood-Werkman formation of C_4 dicarboxylic acids by locating the labeled CO_2 in carboxyls of these acids would not constitute unequivocal proof of *net* synthesis involving carbon dioxide. Evi-

dence has been obtained for the probable operation of a C_4 dicarboxylic acid respiratory cycle in this mold.

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† On leave of absence from the University of Texas.

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OBSERVATIONS ON THE EFFECTS OF MALEIC HYDRAZIDE ON FLOWERING OF TOBACCO, MAIZE AND COCKLEBUR*

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Communicated by E. W. Sinnott, February 7, 1950

In April, 1949, experiments were begun in this laboratory to determine effects of maleic hydrazide[1] on growth of a number of species of plants. Our first objective was to determine its effect upon growth and differentiation of Turkish tobacco plants which were just producing flower buds. Concentrations ranging from 0.05 to 0.8 per cent were used in treating five lots of ten plants each. The solutions were made up in distilled water to which one drop of Aerosol OT was added per 100 ml. to act as a spreading agent. All leaves of the plants were then sprayed on both upper and lower surfaces. Each plant received approximately 25 ml. of the proper solution. Height measurements were made and note taken of the number of flower buds visible without the aid of a hand lens. The plants were then placed on the greenhouse bench in such a manner that light and moisture conditions would be as uniform as possible.

TABLE 1

Effect of Five Concentrations of Maleic Hydrazide on Growth in Height and Flower Expression in Turkish Tobacco

PER CENT MALEIC HYDRAZIDE USED	NO. OF PLANTS TREATED	AVERAGE HEIGHT, CM., APRIL 19	NO. OF PLANTS WITH FLOWER BUDS, APRIL 19	AVERAGE HEIGHT, CM., MAY 17	NO. OF PLANTS WITH LIVING FLOWERS, MAY 17	AVERAGE INCREMENT IN CENTIMETERS
0.8	10	34.6	2	48.2	None	13.6
0.4	10	36.4	3	49.5	None	13.1
0.2	10	38.1	5	57.4	None	19.3
0.1	10	39.4	6	65.0	5	25.6
0.05	10	39.7	4	91.6	10	51.9
Control	10	43.0	1	106.9	10	63.9

None of the plants treated with 0.2, 0.4 and 0.8 per cent maleic hydrazide had produced new flowers and all those initiated at the time of treatment were dead. Some of these abscissed. On the other hand, of the ten plants treated with 0.1 maleic hydrazide, only five produced flowers and these grew slowly. All of those plants receiving 0.05 per cent maleic hydrazide flowered, but again the rate of development of the inflorescences was slower than the controls. Figures obtained on height increment following treatment indicate nearly equal effectiveness of concentrations of 0.2, 0.4 and 0.8 per cent maleic hydrazide in suppressing terminal growth. Lower concentrations showed decreasing effectiveness in inhibiting over-all growth in height (table 1).

Maize is also very markedly inhibited in growth by maleic hydrazide. The effective concentration range for it is approximately the same as for tobacco. Wisconsin hybrid No. 525 maize was used in the tests reported here. Single plants were grown in soil in two-gallon crocks. Seeds were planted in July and the plants were approximately one meter tall with no visible staminate inflorescence (tassel) at the time of treatment. Crystalline maleic hydrazide was used in making the solutions, and Emulphor EL was employed as the spreading agent. All solutions were adjusted to

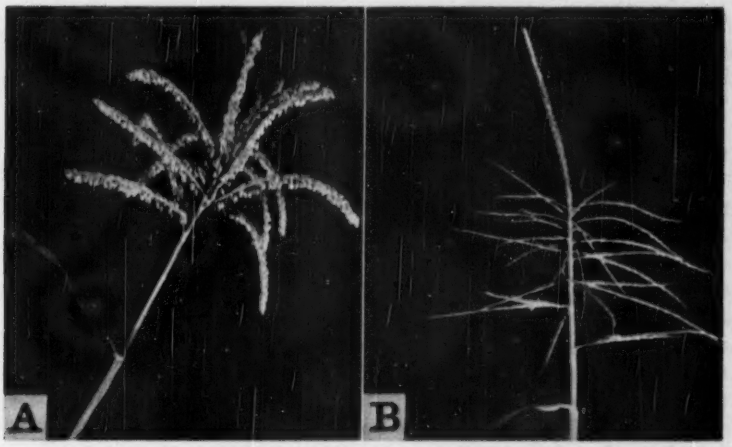

FIGURE 1

Appearance of the staminate inflorescence of maze 45 days after treatment with maleic hydrazide. *A*. Control. *B*. Leaves sprayed with 0.025% maleic hydrazide solution when the plant was 35 days old. Note the lack of anthers in the flowers of the treated plant.

pH 6.0. Concentrations ranging from 0.025 to 0.2 per cent were used. Each one exerted a marked effect.

The highest concentration prevented flower expression completely. The corn plants receiving the lowest concentration grew almost as rapidly as the controls but produced sterile tassels. The number of staminate flowers was approximately the same as in the controls but no anthers were formed (Fig. 1, B). The pistillate inflorescences on these plants, however, were composed of fertile flowers. Seed was set at the base of the ears and more would have probably been set toward the apex had the amount of fertile pollen in the greenhouse been higher.

Because of the striking effects of maleic hydrazide on flower development in tobacco and corn, it seemed desirable to determine if this substance would prevent flowering in a photoperiodically sensitive plant. *Xanthium saccharatum* Wallr.[3] was selected for this purpose because of the large body of information available about light and dark requirements for flowering. Normally Xanthium changes from the vegetative to reproductive condition when provided with cycles of 8.5 hours of dark—15.5 hours of light.[4] Spraying the leaves with as little as 10 ml. of a 0.025 per cent maleic hydrazide solution at the time photoinduction was begun shifted the critical daylength by thirty minutes. Controls which did not receive maleic hydrazide shifted very rapidly from the vegetative to reproductive condition. On the other hand, those receiving this substance changed slowly from the vegetative condition and this held true even when the dark period given was $10^1/_2$ hours long.

Maleic hydrazide thus appears to be a good inhibitor of flower buds as well as vegetative buds.[5, 6] It also causes loss of apical dominance. While it suppresses lateral buds as well as terminals much higher concentrations are required for this than for inhibition of the terminal bud alone. Although some of the leaves produced subsequent to treatment with high concentrations of maleic hydrazide show malformation, concentrations effective in inhibiting flower bud development which do not affect leaf development can be employed. This substance may possibly find application where it is necessary to delay or completely inhibit flowering as in tobacco, and possibly celery and lettuce production. Since there appears to be a difference in sensitivity of terminal and axillary flowers to this substance new possibilities in the production of hybrid seed are opened up. It is often desirable, for example, to have, in hybrid seed corn production, a pollen sterile parent, and preliminary experiments indicate this condition can be induced with the aid of maleic hydrazide.

[2] This work has been aided by a grant from the Rockefeller Foundation.

[1] Supplied through the courtesy of Naugatuck Chemical Company, Naugatuck, Connecticut, as a 30 per cent solution in diethanol amine.

[3] This is the species Hamner and Bonner refer to as *Xanthium pennsylvanicum*. According to Parker, *et al.*,[4] this species has been identified by Dr. S. F. Blake as *X. saccharatum*.

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REPEATED MUTATIONS IN ONE AREA OF A MOUSE CHROMOSOME

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Several years ago it was found that the combination of two different lethal mutations in the house mouse gave rise to a balanced lethal line in which both mutations could be maintained indefinitely in heterozygous condition without selection.[1, 2] This behavior was shown to be due to absence of recombination in this region of the chromosome probably due to allelism of the two mutations concerned. The system can be diagrammed as follows:

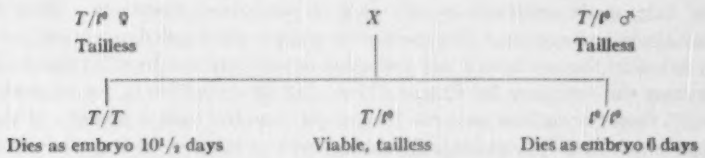

FIGURE 1

Balanced lethal line A.

T symbolizes a dominant mutation, $T/+$ having a short tail (Brachyury) while t^0 is recessive to normal and interacts specifically with *T* to produce the tailless phenotype. Recombination has not been observed either in matings of T/t^0 or of KiT/t^0 or FuT/t^0, *Ki* and *Fu* being dominant mutations about 4 crossover units distant from *T*. The mutation t^0 is thus probably connected with an inversion in this region.[10]

A similar balanced lethal tailless line, T/t^1, known as Line 29 has likewise been maintained for about 15 years.[3, 4] One of the lethals is different from that in Line A, since crosses of T/t^0 by T/t^1 produce, in addition to tailless progeny T/t^0 and T/t^1, a regular proportion of *normal-tailed* offspring which when tested prove to be t^0/t^1. The mutation t^1 likewise was found to eliminate recombination over the area including the loci of *T* and *Ki*. A third line[5] (Line 19) behaved in all respects like Line 29 and contained a lethal, t^2, not distinguishable from t^1.

In inbreeding the balanced lethal lines, T/t^n, rather rare exceptional offspring were occasionally found, about a dozen during fifteen years among several thousand regular offspring. These were saved and tested

as possible evidences of recombination between T and t^n(t^0, t^1, t^2). Recombination in this region should occasionally result in gametes Tt^n and $++$, which in combination with regular gametes T and t^n should give rise to two viable exceptional phenotypes: $T/++$ (Brachyury or short tail) and $t^n/++$ (normal tail).

One exception with a short tail resembling Brachyury proved to be T/t^n combined with other modifying genes for increased length of tail. The latter were later concentrated by selection in a line which bred true to the short-tail condition but which nevertheless proved to be T/t^n (either t^0 or t^1) as in the parents. It contained no new mutation at the T locus.

The normal-tailed exceptions which were tested proved not to belong to either of the recombinant types. All of those which were adequately tested proved to be t^1/t^n, that is, each contained the t-type mutation of the line in which it arose and another different mutation of the same type.

The detailed breeding data on the first of these additional mutations to be extensively analyzed are in course of publication elsewhere. Here it need only be mentioned that whether or not a normal-tailed exception from a balanced line contains a new mutation of this type can be determined by testing the exception by Brachy, $T/+$. If the exception is, for example, t^1/t^n, then two tailless lines can be derived from the tailless progeny of the test cross, i.e., T/t^1 which will balance both in inter se matings and when tested by the parent type T/t^1; and T/t^n, a new tailless line which will always give normal-tailed progeny t^1/t^n when crossed with the parental line. Whether the new mutation gives rise to a new balanced lethal line will depend on whether it is (1) lethal when homozygous and (2) fails to undergo recombination with locus T. One of the newly recognized mutations, t^3, is viable when homozygous, so that matings of tailless T/t^3 by tailless T/t^3 regularly yield tailless T/t^3 and normal (or nearly normal-tailed) progeny which can be shown to be t^3/t^3. Another exception has given rise to a new balanced lethal line T/t^4, in which t^4/t^4 dies before birth. Neither t^3 nor t^4 have shown recombination with T.

In addition to these, we have recorded other exceptions of similar origin, i.e., found as single normal-tailed individuals in the balanced lethal tailless lines. Exceptions t^6, t^9, t^{11} were found in Line A (T/t^0) but the chromosomes containing them were lost before it could be determined whether they represented new t alleles or recurrences of old ones such as t^1. Exceptions t^2, t^4, t^5, t^7, t^8, t^9 and t^{10} were found in the balanced Line 29 (T/t^1). Exception t^8 was found in a nearly sterile male (t^1/t^8) and the chromosome was lost in F_2 following outcross. Exceptions 3, 4, 7, 8 and 9 were found in normal-tailed females which segregated for t^1 and another allele without recombination (t^1/t^n). Lines with the new alleles are being extracted. Exception t^{10} was found in a normal-tailed male from T/t^1 parents and is being tested.

In addition to these occurrences in our laboratory, Dr. T. C. Carter, Institute of Animal Genetics, Edinburgh, Scotland, has found tailless mice in a normal stock of mixed ancestry having no known relationship to T or t^n stocks. These proved to contain a new mutation[6] indistinguishable from T, and a t-type mutation (t^6) which is lethal. These have given rise to a new balanced lethal tailless line, Line 6 (T/t^6). Using animals kindly sent to us by Dr. Carter, we have found t^6 to be different from t^1 since t^1/t^6 is viable and normal. No combinations t^0/t^6 have yet been found (from crosses of $T/t^0 \times T/t^6$) and so t^6 is not distinguishable from t^0 and may be a recurrence of t^0.

It is apparent that inbreeding of balanced lethal lines provides an excellent method for detecting changes of the above sort and that the region involved is in fact quite mutable. We estimate that a minimum of 14 changes of the t type have occurred together with two of the T type. Earlier indications of what may have been similar changes[7, 8] are not included. The mode of origin of these changes is interesting, since in our laboratory all have been found in the offspring of parents who themselves contained a t-type chromosome (3 from T/t^0, 7 from T/t^1). We have tested many sporadic tailless mice found in our Brachy $T/+$ stocks and in outcrosses, but have never found a t-type mutation in these. The first t^0 mutant was found by Dobrovolskaia-Zavadskaia[9] after outcross of $T/+$ by a wild mouse; and t^1 and t^2 were found after outcrosses of $T/+$ to normal laboratory stocks, but since the $T/+$ stocks of Zavadskaia occasionally produced tailless animals, the prior existence of t-type chromosomes in her stocks cannot be excluded.

It is probable that t^0 and t^1 are connected with inverted sections of chromosomes IX since they prevent recombination in the region from T to *Ki*—about 4–6 units.[10] The question thus arises whether all those derived from t^1, (t^3, t^4, t^5, t^7, t^8, t^9, t^{10}) contain the same inversion; or whether new inversions have been induced by t^1. This point is being investigated by cytogenetic methods. Unfortunately such a study cannot be made of exceptions derived from t^0 since the few which were detected were lost.

Although the results of such a study need not be anticipated, it is simpler for the present to assume that inversion is a less frequent process than the occurrence of new alleles within an inversion, particularly since loci may become unstable in or near an inversion; and to treat the series $T \ldots t^n$ as allelic alterations of the same locus. All t mutations, it must be remembered, interact specifically with T to produce taillessness, but show no interaction[10] with the nearby loci *Ki* and *Fu*. They have certain other peculiarities in common also such as the production of male sterility[11] in compounds t^n/t^x which indicate similar interaction effects upon spermatogenesis. Extensive fertility tests on males t^0/t^2 and t^1/t^2, which will

be reported separately from this laboratory, and tests on other alleles now in progress, show that while t^6/t^z males are most often sterile, some do produce functional sperm. Such semisterile males show the same absence of recombination as occurs in t^6/t^z females. Whether the sterility is influenced chiefly by the particular alleles or by the inversions concerned remains to be determined. The male sterility is important not only as evidence of interaction among alleles but as a limitation to the recovery of new alleles which in general can only be recovered from female exceptions.

Although in these respects alterations of the *T* "locus" conform to those expected from alleles, there is one way in which they are quite exceptional. So far as tested the compounds containing two *different* alleles are viable even when the component alleles are lethal when homozygous. This is true of t^6/t^1, t^6/t^2, t^6/t^4, and t^1/t^4, i.e., all cases so far thoroughly tested in which both alleles are known to be lethal. As pointed out in the first description of this phenomenon of complementary action of alleles,[1] this means that there is a physiological divergence of function such that one lethal allele makes good the defect brought about by the other. This type of divergence is difficult to reconcile with any simple hypothesis of quantitative alterations of the same single element. Either several elements, each susceptible of separate alteration and of influencing different processes are held together at such a locus in an organization that is not disrupted by crossing over,[12] or else the single element is capable of a very large variety of changes leading to physiologically antithetic or compensatory changes.

Several cases of such complex loci have recently been shown to consist actually of two or more loci separable by rare crossing-over[13, 14, 15] and forming "pseudoallelic series." Other allelic series are known within which apparently qualitative changes have occurred, requiring the assumption that the gene or locus consists of more than one element.[16] This situation apparently obtains at the loci governing self-incompatibility in plants[17] and possibly at the Rh locus in man.[18] Finally, there are modifications of what appears to be a single locus which show related effects and dominance in relation to one character and compensatory or antithetic effects upon another.[19, 20] Hosino has in fact provided an exact parallel to the relations of *t* alleles since in the ladybird beetle he has found over 20 alleles governing elytral pattern, among them two which are lethal when homozygous but viable in compound.[20] Lewis, similarly, has shown that a self-incompatibility gene in *Oenothera organensis* may exhibit two antithetic types of specificity, and assumes that this locus consists of at least two parts.[17] These cases indicate that there may be a more or less continuous gradation of loci from those subject only to the quantitative type of change usually associated with true allelism to those in which

there are several separable elements, which have remained together after adjacent duplication or repeat formation. The *T* locus in the mouse probably belongs in about the middle of this spectrum together with the *S* locus in plants and the spotting locus in the ladybird beetle; that is, the "alleles" show rather wide physiological divergence and complementary interactions in certain of their effects, yet share similarities indicative of allelism and show no recombination.

* We are very grateful to Dr. T. C. Carter, Institute of Animal Genetics, Edinburgh, Scotland, for sending to us stock of a new mutant type discovered by him and for permission to refer to some of his unpublished observations about it.

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SPECIFICITY OF INTERACTION BETWEEN IDENTICAL MOLECULES

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Among specific interactions between molecules, there is one type which is of particular importance, and that is the specific attraction between a pair of macromolecules on condition that they are identical or almost identical. Such a phenomenon is encountered in synapsis during meiosis, conspicuously in the phenomenon of inverted synapsis, which shows that pairs of almost identical genes attract each other strongly.

The most striking example of specific attraction between identical large molecules occurs in the self-duplication process of genes, i.e., of chromosomes in mitosis. The current view about self-duplication is as follows. Starting with an original chromosome thread, one assumes that replicas of the genes contained in that chromosome are gradually being built up from the material of the cell medium. By selective attraction, the constituent molecules of the original gene collect, from the surrounding medium, molecules which happen to be identical with the former ones (and which because of their smallness compared with a gene, are readily available in the cell), to the neighborhood of the corresponding molecules of the original gene. Thereafter, chemical bonds link these molecules together, forming a daughter gene. What is so astonishing in this phenomenon is its accuracy of differentiation, which permits identical replicas to be handed down through enormous numbers of chromosome generations; its versatility, which implies that all kinds of genes and their mutants self-duplicate accurately; and the long range of the specific forces involved, just as in synapsis. The accuracy of the phenomenon is evident; any mistake in self-duplication in the sex cells would show up as a mutation, in addition to those mutations[1] which are caused by a monomolecular change of the genetic material due to radiation or temperature effects. The versatility is evidenced by the inheritability of mutations: The fact that mutated genes are accurately reproduced shows in a striking fashion how much the self-duplication phenomenon is independent of the particular molecules involved. The long range of the specific interaction forces is indicated by the fact that the pair of specifically interacting molecules will have to interact at distances at least equal to the considerable thickness of the constituent molecules and they are usually not flat. Therefore, we have to look at these phenomena as something very different from ordinary chemical bond formation.

Various theories have been advanced to explain self-duplication.[2] The duplicates will have to be assumed to be actual replicas, rather than but functionally similar molecules; otherwise, it will be an insurmountable difficulty to account for the accuracy and versatility of the phenomenon. In this respect, self-duplication of genes is quite different from other building processes in biology.

Many attempts have been made to explain specific interaction on the basis of steric effects. Again, the accuracy of specific attraction, the versatility and range of the phenomenon would be very difficult to understand on such a hypothesis. Also we would have to expect the formation of complementary structures, rather than replicas of genes: A laevo structure would be expected to build up a dextro structure.

It has been shown[3] that some special compounds can be understood to reproduce by the assumption of a chemical chain reaction process. The serious difficulty of such a theory is the impossibility to account for the versatility with which all types of genes perform the same accurate reproduction.

H. J. Muller[4] suggested the investigation of interaction of thermally excited molecule vibrations. Molecules in whose specific interaction we are interested have a well-defined structure: X-ray investigations of some proteins which are supposedly similar to constituent molecules of genes have shown such large numbers of Fourier components that even without knowing their structure we know that every atom is located at a well-defined place.[5] Also, the following argument may support this view of a rigid structure. Organic molecules in the cell usually form and break bonds at a considerable rate. It is only molecules with well-defined structures (without possibilities of many internal rotations) which have a high probability that any broken bond will properly heal up again, without some foreign molecules getting a chance to join in at a break. Thus, a necessary condition for the outstanding stability of the genes is that the material of which the genes are built have a rigidly defined structure, say, as in a three-dimensional framework. The amplitudes of the vibrations are of the order of 10^{-9} cm., a figure which depends but little upon whether we consider the lowest or highest vibrations in the molecule. The highest wave numbers are well above $kT/hc \sim 200$ cm.$^{-1}$, that is in the domain where quantum effects are important. We know that molecular vibrations are usually but little perturbed by the environment in which the molecule is placed. Let us study coupled thermal vibrations of a pair of identical molecules and investigate them as a possible cause of their specific attraction. As concerns the interaction mechanism between the pair of molecules, no exponentially decreasing short range force, e.g., chemical bonds, can be responsible. Neither can it be a type of force with considerable relaxation time, as, e.g., Debye-Hueckel forces. Neither can it be a force

transmitted by the molecules of the medium in between the pair of molecules, because Brownian motion of the medium would jam the interaction. Thermally excited molecular vibrations are accompanied by vibrating electrical dipole moments which interact instantaneously for all practical purposes at short distances between the pair.

Let us first mention how specific attraction cannot be produced. Consider, for simplicity, a pair of harmonic vibrators, e.g., two negatively charged mass points which can vibrate about two positive centers only along the line connecting them with displacements z_I, z_{II}. R is the distance between the centers. The partition function in *classical* statistical mechanics

$$Z = \int\int \exp(-T/kT)dp_{zI}\,dp_{zII} \int\int \exp(-V/kT)dz_I\,dz_{II} \quad (1)$$

$$T + V = {}^1/_2(m_I^{-1}p_{zI}^2 + m_{II}^{-1}p_{zII}^2) + {}^1/_2(k_I^2z_I^2 + k_{II}^2z_{II}^2) - 2e^2R^{-3}z_Iz_{II}$$

will contain the masses m_I, m_{II} only through the first double integral, that is as factors in Z. Thus, the attractive force $-kT\partial \log Z/\partial R$ is independent of the masses, that is, independent of whether the frequencies of the pair are identical or not. The same holds if each molecule is represented by a set of vibrators, harmonic or anharmonic.

Quantum mechanical resonance of a pair of *simple harmonic* vibrators will not lead to a specific attraction either. Let the z direction be along the line connecting the two molecules. The molecules' orientation with respect to the axes may be described by stating that the components of the electric dipole moments of the two vibrators I, II are

$$\mu_{xI}q_I,\ \mu_{yI}q_I,\ \mu_{zI}q_I,\ \mu_{xII}q_{II},\ \mu_{yII}q_{II},\ \mu_{zII}q_{II},$$

where $q_I = m^{1/2}z_I$ if orientated in z direction. We consider first the interaction for a given, fixed, mutual orientation of the molecules; the ratios of the μ's are regarded as parameters which describe orientation effects to be discussed later. With the Hamiltonian ($\kappa_I = k_I/m^{1/2}$)

$$H = {}^1/_2(\dot{q}_I^2 + \dot{q}_{II}^2) + {}^1/_2(\kappa_I^2q_I^2 + \kappa_{II}^2q_{II}^2) + R^{-3}[\mu_{xI}\mu_{xII} + \mu_{yI}\mu_{yII} - 2\mu_{zI}\mu_{zII}]q_Iq_{II} \quad (2)$$

we find for the classical frequencies ω_+ (inphase mode) and ω_- (antiphase mode)

$$\left.\begin{aligned} \omega_\pm^2 &= {}^1/_2(\kappa_I^2 + \kappa_{II}^2)(1 \pm \epsilon); \\ \epsilon &= \{(\kappa_I^2 - \kappa_{II}^2)^2 + 4R^{-6}[\mu\mu]^2\}^{1/2}/(\kappa_I^2 + \kappa_{II}^2) \end{aligned}\right\} \quad (3)$$

where $[\mu\mu]$ is the square bracket of equation (2) and the signature of the square root in the expression for ϵ has to be taken equal to the signature of $[\mu\mu]$. Figure 1 shows this well-known behavior of the proper frequencies, the vertical lines representing the influence of the coupling. Note the lack of sharpness of this resonance effect: Far away from $\kappa_I = \kappa_{II}$ we still have a strong influence of resonance.[2]

We chose interacting *harmonic* vibrators because the quantum levels can be simply expressed in terms of ω_+ and ω_-. The dipole-dipole interaction between the pair results in level shifts, upwards and downwards, which are strongest if the two vibrators have identical frequencies $\kappa_I = \kappa_{II}$. But the principal term of $\partial \log Z/\partial R$ turns out to be independent of whether the vibrators have identical frequencies or not; anharmonicity of the vibrators will not change this fact. To show this let us form the partition function Z for the set of levels shown in figure 2.[6] We see immediately from this figure that n_+ predominates over n_- among the thermally accessible quantum states, i.e., we get attraction. A simple calculation gives

$$\log Z = -\{{}^1/_2\hbar\omega_+/kT + \log(1 - \exp(-\hbar\omega_+/kT)) + {}^1/_2\hbar\omega_-/kT + \log(1 - \exp(-\hbar\omega_-/kT))\}. \quad (4)$$

In the classical limit $\hbar\omega_\pm/kT \ll 1$

$$\partial \log Z/\partial R \to {}^1/_2(1 - \epsilon^2)^{-1}\partial\epsilon^2/\partial R \quad (5)$$

whereas in the upper limit $\hbar\omega_\pm/kT \gg 1$

$$\partial \log Z/\partial R \to \frac{1}{8}(1 + \frac{5}{8}\epsilon^2)\left(\frac{\kappa_I^2 + \kappa_{II}^2}{2}\right)^{1/2}\frac{\hbar}{kT}\frac{\partial\epsilon^2}{\partial R} \quad (6)$$

The expression (3) for ϵ shows that the term $(\partial\epsilon^2/\partial R)$ does not depend upon whether $\kappa_I^2 - \kappa_{II}^2$ is small or not. This demonstrates the insensitivity of attraction of a pair of simple vibrators with respect to their frequencies being identical or not. The same holds if we use higher approximation in evaluating (6).

Quite a new situation arises if we consider quantum mechanical resonance between a pair of molecules, each of them having a *number of vibrational modes;* and if we take account of the anharmonic terms in the potential energy of vibration of each of the molecules. Let us consider first one molecule as a set of vibrators *which are coupled because of anharmonic terms*. Although we can get rid of quadratic cross-product terms by introducing "normal coordinates," the presence of anharmonic terms means that we certainly cannot abolish cubic and higher cross-product terms in the potential energy, and those will cause coupling between the "modes" of a molecule. In a complicated molecule there will be many "modes" which are almost commensurable (that is, much accidental degeneracy and resonance) which causes strong level shifts. In correspondence terms, certain phase relationships between the classical phases of the almost commensurable modes of one molecule will have lower energy and, therefore, because of the Boltzmann factor, be statistically preferred—the more preferred, the stronger the anharmonicity. This quantum effect depends on h/T.[7]

For simplicity, consider first the case of strict phase locking between the modes of each one of the molecules, instead of just phase preferences. This case is approached if there are strong anharmonicities and if there are many commensurabilities in the molecule. Let us then consider a pair of anharmonic molecules, each with phase-interlocked vibrations and the pair coupled by ordinary electric dipole-dipole interaction. If the lowest of the commensurable modes of molecule I is inphase with the lowest of II (regarding their z components), and if the molecules are identical, the other commensurable modes will be pairwise inphase also, which amounts to a strong negative interaction energy and thus a big contribution toward

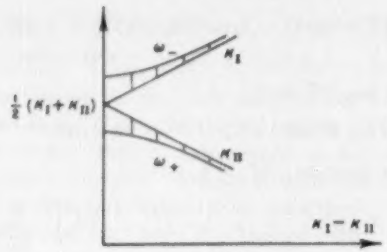

FIGURE 1

Inphase and antiphase frequencies in the vicinity of resonance.

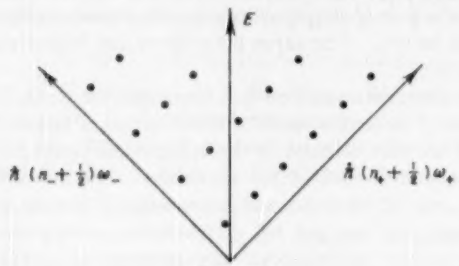

FIGURE 2

Example of thermally accessible quantum states.

the partition function. If the lowest modes of the two molecules are antiphase, the vibrations contribute little to the partition function. Such a pair of molecules behaves like a pair of vibrators with very large vibrational amplitudes; we may apply again the figure 2 as representing the thermally accessible quantum states of the pair of molecules only that higher statistical weights will have to be attributed to higher quantum states. More precisely: A state $n_+ = 4$, $n_- = 1$ which indicates a strong

excitation of the inphase mode and a weak excitation of the other mode will, in this picture, have the same statistical weight as a state $n_+ = 1$, $n_- = 4$; the statistical weights will very strongly increase with higher quantum numbers of this representative pair of large amplitude vibrators. Thermal accessibility on the other hand, can be represented by a horizontal line $E = \text{const.}$, limiting the region of accessible states. In consequence, the thermal excitation of the inphase mode will be overwhelming compared with that of the antiphase mode, i.e., the number of thermally excited states in which n_+ dominates over n_- is much larger than those in which $n_- > n_+$. The net effect of the contributions of all quantum states amounts to a decrease of the free energy, that is an attraction.

If we consider phase preferences instead of strict phase locking inside the molecules, we will get a similar effect but of smaller order. In the classical limit $h/T \to 0$, resonance effects and specific attraction disappear. Clearly this attraction is specific with respect to the two molecules being structurally and therefore vibrationally identical, as the phase preferences within one molecule will match those within the other only provided that the molecules are identical and properly orientated.

Equation (2) tells us about orientation effects. Consider two identical molecules in the same mutual orientation as are two identical closed dictionaries which lie right on top of each other, each dictionary's front cover facing upward, but the bindings facing in opposite directions. Let the z axis in this illustration be vertical, i.e., in the direction connecting the centers of the two molecules (dictionaries). If each of the molecules vibrates *en bloc* with established phase relationships and if the pair of molecules vibrates in synchronism, we have a form of motion in which the z_{I}, z_{II} components of vibration all vibrate, e.g., inphase and therefore, all x_{I}, x_{II} components and all y_{I}, y_{II} components vibrate in antiphase. So all three components contribute an attractive interaction potential. Such a peculiar kind of mutual orientation is caused by the same effect as the attraction itself; the pair has lower energy if orientated in that way; Brownian motion will bring about this orientation.

The orientation effects in the interaction-term of (2) will cause a molecule with laevo configuration to have a strong interaction but with another laevo molecule; the same a dextro with a dextro, as a short consideration of various orientations readily shows. This accounts for the fact that a laevo structure always builds up a laevo structure, and that an admixture of some dextro will cause considerable trouble with respect to its acceptability.

After a pair of molecules has come close together, in the energetically most favorable orientation, the dipole field of the pair is altogether different from that of a single molecule which prevents the pairs field from matching the field of a third identical molecule, thereby failing to attract the third.

So triplets are not formed. Another pair may, however, be attracted; so we may expect formation of pairs, quadruplets, octuplets, etc.

We shall expect this specific attraction effect to occur on various scales, probably from small peptide rings up to larger constituent molecules of genes, to genes and gene-complexes and correspondingly for viruses.

A qualitative argumentation as this one is more easily carried through in terms of Bohr's formulation of quantum theory[8] and the correspondence principle rather than by straight quantum mechanics.

Because of the complications of straight-forward quantum mechanical calculations of a pair of identical molecules with commensurable modes, we simplify the model, and show that the above-mentioned effects exist. Anharmonicity strongly couples commensurable modes; quantum mechanically, anharmonicity brings about level splitting (level shifts) between various (e.g., symmetric or anti-symmetric) combinations of resonating ψ functions. These level shifts are the stronger, the stronger the anharmonicity, the closer the commensurability and the larger the number of resonating modes.

Interactions between "modes" in a molecule I exist only if there is some anharmonic perturbation function, e.g. $\lambda q_{1I}^{2} q_{2I}$ in case of commensurability 1 to 2 between the modes, or other more complicated perturbation functions. Similarly for other commensurability ratios, and for the molecule II. The effects, discussed above in terms of the correspondence principle do, however, not much depend on the kind of coupling which is assumed to exist in between the modes of each one of the molecules. Therefore, in our simplified crude model we represent this situation by an interaction $\lambda q_{1I} q_{2I}$ and $\lambda q_{1II} q_{2II}$ of two exactly equal frequency harmonic pseudomodes inside each of the identical molecules I and II. We assume q_{1I} and q_{2I} parallel orientated and λ negative. As we look for a quantum effect we assume that, besides the groundlevel 0, only the next bunch of levels 1 is thermally excited. A detailed calculation showed that for the effect under consideration only first-order perturbation theory needs to be applied. We assume a simple perturbation function

$$H_1 = \lambda(q_{1I} q_{2I} + q_{1II} q_{2II}) + \mu(q_{1I} q_{1II} + q_{2I} q_{2II} + q_{1I} q_{2II} + q_{2I} q_{1II}) \quad (7)$$

where μ is an expression of the type

$$R^{-3}[\mu_{x1}\mu_{x11} + \mu_{y1}\mu_{y11} - 2\mu_{z1}\mu_{z11}]. \quad (8)$$

H_1 becomes diagonal with the eigenfunctions

$$\begin{aligned}
\psi_{00} &= \psi_0(q_{1I})\psi_0(q_{2I})\psi_0(q_{1II})\psi_0(q_{2II}) = (0000) \\
\psi_{10} &= {}^1/_2\{(0100) + (1000) + (0001) + (0010)\} \\
\psi_{11} &= {}^1/_2\{(0100) + (1000) - (0001) - (0010)\} \\
\psi_{12} &= {}^1/_2\{(0100) - (1000) + (0001) - (0010)\} \\
\psi_{13} &= {}^1/_2\{(0100) - (1000) - (0001) + (0010)\}
\end{aligned} \quad (9)$$

In order to show how dispersion forces fit into this scheme, we calculate the ground level up to second order perturbation terms (using perturbations due to levels up to the second bunch).

$$\begin{aligned} E_{00} &= (4 - (\lambda^2 + 2\mu^2)/2\kappa^4)^{1}/_{2}\hbar\kappa \\ E_{10} &= [6 + (\lambda + 2\mu)/\kappa^2]^{1}/_{2}\hbar\kappa \\ E_{11} &= [6 + (\lambda - 2\mu)/\kappa^2]^{1}/_{2}\hbar\kappa \\ E_{12} &= [6 - \lambda/\kappa^2]^{1}/_{2}\hbar\kappa \\ E_{13} &= [6 - \lambda/\kappa^2]^{1}/_{2}\hbar\kappa \end{aligned} \tag{10}$$

This shows the statistical preference of the "inphase" mode ψ_{10} over the three other excited ψ_{11}, ψ_{12}, ψ_{13}. The negative of the attractive force becomes

$$kT\partial \log Z/\partial R = kTZ^{-1}(\partial Z/\partial\mu)(d\mu/dR) \sim kT\frac{d\mu}{dR}\exp\frac{2\hbar\kappa}{kT}\cdot$$

$$\left[1 - 4\exp\left(-\frac{\hbar\kappa}{kT}\right)\right]\cdot\frac{\partial}{\partial\mu}\left[\exp\left(-\frac{E_{00}}{kT}\right) + \exp\left(-\frac{E_{10}}{kT}\right) + \exp\left(-\frac{E_{11}}{kT}\right) + \cdot + \cdot\right] \sim kT\frac{d}{dR}\left(\frac{\mu}{\kappa^2}\right)\cdot$$

$$\frac{\mu}{\kappa^2}\frac{\hbar\kappa}{kT}\left[1 - 4\exp\left(-\frac{\hbar\kappa}{kT}\right) + 2\frac{\hbar\kappa}{kT}\exp\left\{-\left(1 + \frac{1}{2}\frac{\lambda}{\kappa^2}\right)\frac{\hbar\kappa}{kT}\right\}\right] \tag{11}$$

This shows that the effect is a quantum effect and that it is proportional to R^{-7}, cf. (8). The effect also depends on the factor

$$\exp\left(-\frac{1}{2}\frac{\lambda}{\kappa^2}\frac{\hbar\kappa}{kT}\right) \tag{12}$$

i.e., it depends exponentially on the strength $(-\lambda)$ of coupling in between the modes of each one of the molecules. The first and second term in the last square bracket in (11) originates from $\partial E_m/\partial\mu$ and represents London's dispersion forces. A similar calculation for a pair of molecules, each one containing n equal modes, shows a much stronger influence of λ of the same character; in a big molecule the factor corresponding to (12) will be large compared with one, it becomes $\exp\left\{-{}^{1}/_{2}(n-1)\lambda\kappa^{-2}\hbar\kappa/kT\right\}$.

In order to study the specificity of attraction in the case of our simplified model, we applied a small detuning δ, $(\kappa^2\delta^2 \gg \lambda^2)$ and calculated its influence on the λ term (12) of the force (11). The case $\kappa_{1I} = \kappa_{2I} = \kappa + \delta$, $\kappa_{1II} = \kappa_{2II} = \kappa - \delta$ brings, like (5) and (6), no change, i.e. again (11). The case of $\kappa_{1I} = \kappa_{1II} = \kappa + \delta$, $\kappa_{2I} = \kappa_{2II} = \kappa - \delta$ makes the term (12) disappear, so does the case $\kappa_{1I} = \kappa + \delta$, $\kappa_{1II} = \kappa - \delta$, $\kappa_{2I} = \kappa_{2II} = \kappa$, and also the case $\kappa_{1I} = \kappa + \delta$, $\kappa_{2I} = \kappa - \delta$, $\kappa_{1II} = \kappa_{2II} = \kappa$. In other words, the attraction is specific with respect to the latter three changes.

This is the effect of phase preferences on the attraction. Our former argumentations make it evident that in the case of commensurable rather than equal modes the effect of *identical* phase preferences in the pair of molecules which depends on the identity of the pair, is causing the specificity.

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*INHERITANCE OF SEXUALITY IN CHLAMYDOMONAS REINHARDI**

By Gilbert M. Smith and David C. Regnery

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Moewus[1-5] has studied the inheritance of sexuality in haploid heterothallic (dioecious) algae where the zygote nucleus divides meiotically and where a germinating zygote produces four haploid cells in which two of the cells are of one sex and two of the opposite sex. He has analyzed sexuality of cells from germinating zygotes of five heterothallic species of *Chlamydomonas* and one heterothallic species of *Polytoma*. For all species investigated he finds exceptional cases in which all cells produced by a germinating zygote are homothallic (monoecious) instead of all being heterothallic. For zygotes produced by union of male and female cells of the same species he found that the percentage of exceptions ranged between 2.8 and 7.8. In germinating zygotes formed by a cross between *Chlamydomonas paradoxa* and *C. pseudoparadoxa* the percentage of exceptions was 11.7. Moewus holds that the genes for maleness and femaleness are not alleles, but are at different loci on homologous chromosomes, and that as a result of crossing-over one chromosome contains the genes for both sexes and the other chromosome lacks genes for sex. Daughter nuclei lacking genes for sex disintegrate; those with genes for both sexes persist and cells with such nuclei are homothallic.

Plus and minus (male and female) clones of several species of *Chlamydomonas* have been isolated at Stanford University. These have been cultured in an immobile palmelloid condition on 0.1% Beijerincks solution plus 1.5% agar. When palmelloid cultures growing on agar are flooded with distilled water the cells develop flagella and become motile shortly after flooding. If motile cells from clones of opposite sex are mixed there is an immediate fusion in pairs to form zygotes. The resultant zygotes become thick-walled within a few days, and are fully ripened in about three weeks. Germination of ripe zygotes may be induced by placing them on nutrient agar.

Of the species isolated at Stanford, *C. Reinhardi* proved the most satisfactory for study of inheritance of sexuality. It has relatively large zygotes; germinates 22–26 hours after transfer to nutrient agar; and about 90% of the zygotes germinate. Single zygotes of *C. Reinhardi* were isolated on blocks of agar about 5 mm. square. Four blocks, each with a single zygote, were quadrately arranged 5 cm. from one another on nutrient agar in a Petri dish. At the end of 26 hours each of the small blocks was flooded with a large drop of water. This induced motility in daughter cells extruded from germinated zygotes and the cells swam about in the small puddle of water. Swimming continued until the motile cells became stranded on the agar surface because of absorption of the water by the agar. Each stranded cell, by division, developed into a colony that at the end of ten days contained hundreds of cells. When the colonies were large enough to be visible to the naked eye, each was fished out with a sterile needle and transferred to an agar slant. The sexuality of the four or eight clones obtained from each germinated zygote was then determined by flooding with distilled water and noting the sexual reaction of the cells made motile by flooding.

The sexual reaction was determined for each of the clones developed from individual cells liberated from 249 zygotes of *C. Reinhardi*. None of these clones proved to be homothallic. A few (17) isolates did give a homothallic reaction when tested for sexuality. However, when clones were reisolated from vegetative cells of these putative homothallics all proved to be heterothallic and not homothallic. From this it is evident that the homothallic reaction of the original isolation was due to a heterothallic colony being invaded by cells of opposite sex.

All clones proved to be heterothallic and, where four or eight clones were obtained from a zygote, with half the clones of one sex and half of the opposite sex.

In view of the percentage of crossing-over of genes for sex reported by Moewus for five species of *Chlamydomonas* it is reasonable to expect that at least one case would have been found for *C. Reinhardi*. Since this has not been found in *C. Reinhardi* it is very probable that in this species the

genes for sex are at the same locus and not at different loci as Moewus finds in the species he investigated.

For inheritance of sex, as well as for inheritance of other characters, Moewus always reports obtaining four clones from a zygote. His papers do not show whether he always obtained four clones or whether he discarded material from zygotes where he obtained fewer or more than four clones. Germinating zygotes of *C. Reinhardi* may produce eight instead of four cells, and the number of zygotes producing eight cells is greater in old than in recently ripened ones. Although there is always a liberation of four or eight cells, there is not always a development of four or of eight colonies. For example, in a random selection of 100 zygotes recorded as liberating four cells, 83 had a development of four colonies, 11 a development of three colonies, and 6 a development of two colonies. The reason why certain liberated cells fail to divide and develop into colonies is as yet undetermined, but it is not correlated with sexuality. When the three colonies developed from a single zygote are tested for sexuality two are always of one sex and the third is of the opposite sex. When there is a development of but two colonies the two may be of the same sex, and either plus or minus, or one colony may be plus and the other minus.

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THE HOMOLOGY STRUCTURE OF SPHERE BUNDLES

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1. Since a fiber bundle is a generalization of a product space, it is natural to expect some relations between the homology groups of the bundle, the base space, and the fiber. The simplest relation is between the Euler characteristics, the characteristic of the bundle being the product of the characteristics of the base space and the fiber. For sphere bundles the first comprehensive result was obtained by Gysin.[1] Recently, Steenrod[2] gave a new derivation of the Gysin results. On the other hand, results have been announced by Hirsch[3] and Leray[4] for more general types of bundles.

In the works of both Gysin and Steenrod the base space is assumed to be an orientable manifold. The main purpose of our work is to extend

this result to the case that the base space is a complex and to furnish a simpler and more direct method of approach which can be applied to more general fiber bundles. The principal theorem is a statement that a certain sequence of homomorphisms of cohomology (or homology) groups is exact. It seems to us that this theorem includes all known results about the cohomology structure of sphere bundles. As a basis of our treatment we make use of the axiomatic homology theory of Eilenberg and Steenrod.[5]

In §2 we define the type of bundle to be studied. In §3 we state the main results. Some applications of these results are given in §4, including a proof of Gysin's main theorem and a derivation of some relations between the Betti numbers of the bundle, the base space and the fiber. An indication of the methods used is given in §5.

2. Let K denote a finite connected simplicial complex of dimension n. Let $B = |K|$ be the space of K, and let X be a topological space. A continuous map $f:X \to B$ is called a *fibering of X into d-spheres over B* if for each simplex s of K there exists a homeomorphism

$$\varphi_s:|s| \times S^d \approx f^{-1}(|s|),$$

where S^d denotes the d-dimensional sphere, such that:

(*a*) $f\varphi_s(y, z) = y \qquad$ for $y \in |s|$, $z \in S^d$.

(*b*) If s is a face of s', then

$$g_{ss'}(y):S^d \approx S^d$$

defined by

$$\varphi_s(y, g_{ss'}(y)(z)) = \varphi_{s'}(y, z), \qquad \text{for } y \in |s|,\ z \in S^d$$

is a continuous mapping of $|s|$ into the group of homeomorphisms of S^d of degree $+1$.

X is called the *bundle*, and B is called the *base space* of the bundle. The mapping f is called the *projection* of the bundle. The projection induces homomorphisms

$$f^*:H^p(B) \to H^p(X)$$

of the cohomology groups of B into those of X over any coefficient group. In the following the coefficient group is arbitrary but fixed for all cohomology groups under consideration.

3. Using the bundle structure two homomorphisms will be defined. The first homomorphism maps the cohomology groups of X into those of B and lowers dimension by d. It is denoted by

$$\Phi:H^p(X) \to H^{p-d}(B).$$

This homomorphism has been considered by Gysin and Lichnerowicz[6] in the case when the base space is an orientable manifold.

The second homomorphism maps cohomology groups of B into cohomology groups of B and raises dimension by $d + 1$. It will be denoted by

$$\Psi: H^p(B) \to H^{p+d+1}(B).$$

These two homomorphisms together with f^* form an infinite sequence

$$\ldots \xrightarrow{\Psi} H^p(B) \xrightarrow{f^*} H^p(X) \xrightarrow{\Phi} H^{p-d}(B) \xrightarrow{\Psi} H^{p+1}(B) \xrightarrow{f^*} \ldots$$

called the *cohomology sequence of the bundle.*

The main result is embodied in the following theorem:

THEOREM 3.1. *The cohomology sequence of a sphere bundle is exact.*

This theorem shows that if the homomorphism Ψ can be determined in B then the cohomology groups of X are group extensions of groups defined in B by other groups defined in B. This shows the significance of characterizing the homomorphism Ψ, which can be described as follows:

Any sphere bundle gives rise in a natural way to a unique $(d + 1)$-dimensional cohomology class of B with integer coefficients.[7] This class is called the *characteristic class* of the bundle and will be denoted by Ω. It is the obstruction to the construction of a cross-section over the $(d + 1)$-dimensional skeleton of B. Using the natural pairing of the integers and the abelian coefficient group to the coefficient group, the cup products $u \cup \Omega$ and $\Omega \cup u$ are defined for $u \in H^p(B)$ and are elements of $H^{p+d+1}(B)$. The order of multiplication is immaterial. For odd d this follows simply from the commutation rule of the cup product. For even d it can be shown that $2\Omega = 0$, so that the two products are again equal. Then we have the theorem:

THEOREM 3.2. *For any* $u \in H^p(B)$,

$$\Psi(u) = u \cup \Omega = \Omega \cup u.$$

Naturally there exist dual theorems for the homology groups. In particular, we define homomorphisms

$$f_*: H_p(X) \to H_p(B),$$

$$\Phi': H_p(B) \to H_{p+d}(X),$$

$$\Psi': H_p(B) \to H_{p-d-1}(B).$$

These homomorphisms form an infinite sequence,

$$\ldots \xleftarrow{\Psi'} H_p(B) \xleftarrow{f_*} H_p(X) \xleftarrow{\Phi'} H_{p-d}(B) \xleftarrow{\Psi'} H_{p+1}(B) \xleftarrow{f_*} \ldots,$$

called the *homology sequence* of the bundle. The results for the case of homology are summarized in the following theorem:

THEOREM 3.3. *The homology sequence of a sphere bundle is exact. Moreover, if* Ω *denotes the characteristic cohomology class of the bundle, the homomorphism*

$$\Psi' : H_p(B) \to H_{p-d-1}(B)$$

satisfies the equation

$$\Psi'(z) = \Omega \cap z, \qquad \text{for } z \in H_p(B),$$

4. Gysin's isomorphism theorem is contained in Theorem 3.3, for let $K_p(B)$ be the subgroup of $H_p(B)$ consisting of the kernel of Φ'. Then exactness of the sequence implies that Ψ' induces an isomorphism

$$\bar{\Psi} : H_{p+d+1}(B)/f_* H_{p+d+1}(X) \approx K_p(B).$$

The inverse of this isomorphism is the isomorphism denoted by H by Gysin.

A slightly different isomorphism is obtained for cohomology if we define $K^p(B)$ to be the subgroup of $H^p(B)$ consisting of the kernel of Ψ. Then Φ induces isomorphisms

$$\bar{\Phi} : H^p(X)/f^* H^p(B) \approx K^{p-d}(B).$$

The inverse of $\bar{\Phi}$ is Steenrod's functional cup product.[2] More precisely, if $u \in H^{p-d}(B)$ such that $u \cup \Omega = 0$, then $u \underset{f}{\cup} \Omega$ is defined and belongs to $H^p(X)/f^* H^p(B)$. Then we have

$$\Phi \left(u \underset{f}{\cup} \Omega \right) = u.$$

As a second application we study the influence of the relative position of the fiber in the bundle on the homology structure of the bundle. If S^d denotes a fiber, the inclusion map $i : S^d \subset X$ induces homomorphisms

$$i_* : H_d(S^d) \to H_d(X)$$

$$i^* : H^d(X) \to H^d(S^d).$$

The fiber is said to *bound in the bundle* ($S^d \sim 0$) relative to a coefficient group G if

$$i_* H_d(S^d) = 0$$

where $H_d(S^d)$ is taken over G. In terms of cohomology this condition is known to be equivalent to

$$i^* H^d(X) = 0,$$

$H^d(X)$ being taken over a group dual to G.

To avoid cumbersome statements we assume in the rest of this section that the coefficient group for homology and cohomology is a field.

The groups $H^0(B)$ and $H^d(S^d)$ are both isomorphic to the coefficient field and hence to each other. An isomorphism

$$\nu: H^0(B) \approx H^d(S^d)$$

between them can be found such that

$$\nu\Phi = i^*.$$

Therefore, if $S^d \sim 0$, then $K^0(B) = 0$. This in turn implies that $\Omega \neq 0$ and $k\,\Omega \neq 0$ for any integer k. In particular, if d is even, then $2\,\Omega = 0$ so S^d does not bound in X.

If, conversely, S^d does not bound in X, the argument can be reversed, so we see that $k\,\Omega = 0$ for some integer k. It follows then from the cohomology sequence that there is an isomorphism between $H^p(X)$ and $H^p(B \times S^d)$.

Finally, we shall derive some relations between the Betti numbers of X and B, which were obtained by Gysin, Hirsch, and Leray. Let $\rho^p(X)$ and $\rho^p(B)$ be the pth Betti numbers of X and B and $\rho_0^p(B)$ the dimension of the kernel of Ψ in $H^p(B)$. Then we have, from the cohomology sequence of the bundle,

$$\rho^p(X) = \rho^p(B) - \rho^{p-d-1}(B) + \rho_0^{p-d-1}(B) + \rho_0^{p-d}(B). \qquad (4.1)$$

Let $X(t)$, $B(t)$, $S^d(t)$ be the Poincaré polynomials of X, B, S^d, respectively, and let

$$P(t) = \sum_{p \geq d} (\rho^{p-d}(B) - \rho_0^{p-d}(B))t^p,$$

so that $P(t)$ has non-negative coefficients. It is easily verified by using (4.1) that

$$X(t) = S^d(t)B(t) - (1 + t)P(t),$$

and that

$$[1 - t(S^d(t) - 1)]B(t) \leqq X(t) \leqq S^d(t)B(t),$$

where an inequality between polynomials means a set of inequalities between their corresponding coefficients.

5. We shall indicate in this section the method used in proving the theorems stated in §3.

Let $B^p = |K^p|$ be the space of the p-dimensional skeleton of K. Let $X_p = f^{-1}(B^p)$. Then

$$X = X_n \supset \ldots \supset X_p \supset X_{p-1} \supset \ldots \supset X_0 \supset X_{-1} = 0.$$

Using the fact that $f^{-1}(|s|)$ is homeomorphic to $|s| \times S^d$ for each simplex s of K, it is easy to prove the following

LEMMA 5.1. *Let $f_p:(X_p, X_{p-1}) \to (B^p, B^{p-1})$ be the map defined by f. Then*

$$f_p^*:H^p(B^p, B^{p-1}) \approx H^p(X_p, X_{p-1})$$

is an isomorphism onto. Also there is an isomorphism

$$\lambda_p:H^{p-d}(B^{p-d}, B^{p-d-1}) \approx H^p(X_{p-d}, X_{p-d-1}),$$

while

$$H^q(X_p, X_{p-1}) = \{0\}, \text{ if } q \neq p, p+d.$$

The group of cochains of K, $C^p(K)$, is defined by

$$C^p(K) = H^p(B^p, B^{p-1}),$$

so the lemma shows that $H^q(X_p, X_{p-1})$ is either the trivial group or is isomorphic to a group of cochains of K.

The following diagram will be referred to as the main diagram

$$\begin{array}{ccccccc}
\{0\} & \xrightarrow{\alpha_p} & H^p(X) & & \{0\} & \xrightarrow{\alpha_{p+1}} & H^{p+1}(X) \\
 & & \downarrow \beta_p & & & & \downarrow \beta_{p+1} \\
\cdots & \xrightarrow{\delta_{p-1}} & H^p(X_p, X_{p-1}) & \xrightarrow{\rho_p} & H^p(X_p) & \xrightarrow{i_p} & \\
 & & & & \downarrow \gamma_p & & \\
 & & & & H^{p+1}(X_{p+1}, X_p) & \xrightarrow{\rho_{p+1}} & H^{p+1}(X_{p+1}) \xrightarrow{i_{p+1}} \cdots \\
\cdots & \xrightarrow{\bar{\delta}_{p-1}} & H^p(X_{p-d}, X_{p-d-1}) & \xrightarrow{\sigma_p} & H^p(X_{p-d}) & & \\
 & & \downarrow \tau_p & & \downarrow \bar{i}_p & & \downarrow \gamma_{p+1} \\
 & & \{0\} & & & & \\
 & & & & H^{p+1}(X_{p-d+1}, X_{p-d}) & \xrightarrow{\sigma_{p+1}} & H^{p+1}(X_{p-d+1}) \xrightarrow{\bar{i}_{p+1}} \cdots \\
 & & & & & & \downarrow \tau_{p+1} \\
 & & & & & & \{0\}
\end{array}$$

In the main diagram δ_p and $\bar{\delta}_p$ are coboundary operators and α_p, β_p, γ_p, ρ_p, σ_p, τ_p are induced by inclusion mappings. Using the exactness axiom of cohomology theory and (5.4) the following can be proved.

LEMMA 5.5. *For any $p \geqq 0$, the sequence of homomorphisms α_p, β_p, δ_p, ρ_{p+1}, γ_{p+1}, $\bar{\delta}_{p+1}$, σ_{p+2}, τ_{p+2} is exact.*

The fact that in defining the bundle, g_{zz}, (y) is required to be a homeomorphism of S^d of degree $+1$ furnishes the following results.

LEMMA 5.6. *Commutativity holds in the diagram*

$$\begin{array}{ccc} H^p(X_p, X_{p-1}) & \xrightarrow{\delta_p\sigma_p} & H^{p+1}(X_{p+1}, X_p) \\ {\scriptstyle f_p^*}\uparrow & & \uparrow{\scriptstyle f_{p+1}^*} \\ C^p(K) & \xrightarrow{\delta} & C^{p+1}(K). \end{array}$$

LEMMA 5.7. *Commutativity holds in the diagram*

$$\begin{array}{ccc} H^p(X_{p-d}, X_{p-d-1}) & \xrightarrow{\delta_p\sigma_p} & H^{p+1}(X_{p-d+1}, X_{p-d}) \\ {\scriptstyle \lambda_p}\uparrow & & \uparrow{\scriptstyle \lambda_{p+1}} \\ C^{p-d}(K) & \xrightarrow{\delta} & C^{p-d+1}(K) \end{array}$$

We now proceed to construct the homomorphisms used in forming the cohomology sequence of the bundle from the main diagram.

To compute

$$f^*: H^p(B) \to H^p(K)$$

from the main diagram, let $u \in H^p(B)$. Let $c \in C^p(K)$ represent u. Then $\delta c = 0$, so that $\delta_p\rho_p f_p^*(c) = 0$. Hence there exists $v \in H^p(X)$ such that

$$\beta_p(v) = \rho_p f_p^*(c).$$

It is easy to see that $f^*(u) = v$.

To define the homomorphism

$$\Phi: H^p(X) \to H^{p-d}(B),$$

let $u \in H^p(X)$. Then $\gamma_p\beta_p(u) \in H^p(X_{p-d})$. Since $\tau_p(\gamma_p\beta_p(u)) = 0$, there is $v \in H^p(X_{p-d}, X_{p-d-1})$ such that

$$\sigma_p(v) = \gamma_p\beta_p(u).$$

By (5.3) there is $c \in C^{p-d}(K)$ such that

$$\lambda_p(c) = v.$$

Then

$$\lambda_{p+1}\delta(c) = \delta_p\sigma_p\lambda_p(c) = \delta_p\sigma_p(v) = \delta_p\gamma_p\beta_p(u) = 0,$$

so $\delta(c) = 0$. It follows that c is a cocycle of K and determines a cohomology class of B. This class depends only on u and is defined to be $\Phi(u)$.

To define

$$\Psi: H^{p-d}(B) \to H^{p+1}(B),$$

let $u \in H^{p-d}(B)$. Choose $c \in C^{p-d}(K)$ to represent u. Then $\delta c = 0$, so $\delta_p\sigma_p\lambda_p(c) = 0$. Hence there is $v \in H^p(X_p)$ such that

$$\gamma_p(v) = \sigma_p\lambda_p(c).$$

Now $\delta_p(v) = \bar{v} \in H^{p+1}(X_{p+1}, X_p)$. Let $\bar{c} \in C^{p+1}(K)$ such that $f_{p+1}^*(\bar{c}) = \bar{v}$. Since

$$\delta_{p+1}\rho_{p+1}(\bar{v}) = \delta_{p+1}\rho_{p+1}\delta_p(v) = 0,$$

it follows that $\delta(\bar{c}) = 0$. The cohomology class in B to which c belongs depends only on u and is defined to be $\Psi(u)$.

Having defined the homomorphisms f^*, Φ, Ψ, Theorem 3.1 follows immediately from Lemma 5.5.

If the integers are used as coefficients, let $\omega \in H^0(B)$ be the unit cohomology class in B. It is easy to show that the characteristic class Ω satisfies the equation

$$\Omega = \Psi(\omega).$$

The following is a brief sketch of the proof of Theorem 3.2. We consider $X \times B$ as a bundle over $B \times B$. Let Ψ_1 be the homomorphism Ψ defined for this bundle. Then it can be shown that

$$\Psi_1(\alpha \times \beta) = \Psi(\alpha) \times \beta, \qquad \text{for } \alpha \in H^p(B),\ \beta \in H^q(B).$$

Let $d: B \to B \times B$ be the diagonal map. The induced bundle by d over B is equivalent to the given bundle. It follows from this that

$$d^*\Psi_1 = \Psi d^*.$$

Then

$$d^*\Psi_1(\omega \times u) = \Psi(\omega \cup u) = \Psi(u).$$

But

$$d^*\Psi_1(\omega \times u) = d^*(\Psi(\omega) \times u) = \Psi(\omega) \cup u = \Omega \cup u.$$

Added in proof March 27, 1950. While this was in press an article by Thom appeared in the *C. R. Paris* of January 30, 1950 which essentially contains our theorems 3.1 and 3.2.

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ELIMINATION OF RANDOMIZATION IN CERTAIN PROBLEMS OF STATISTICS AND OF THE THEORY OF GAMES

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1. The main aim of this note is to show that mixed strategies may be eliminated from statistical decision rules based on the observation of random variables with continuous distribution functions, and from games of a similar structure.

After stating a general measure-theoretic result in 2. we deduce in 3. and 4. some results on games. In 5. and 6. we give some results on decision problems. Our methods lead to considerably more general results than those presented here (in particular they can be applied to what may be termed sequential games). The selection of results presented here was motivated by our interest in statistical problems. Proofs and a more complete study of the subject will be published elsewhere.

2. Let $\{x\} = X$ be any space and $\{S\} = \mathcal{S}$ a Borel field of subsets of X. Let $\mu_k(S)$ $(k = 1, 2, \ldots, p)$ be a finite number of real-valued, bounded and countably additive set functions defined for all $S \in \mathcal{S}$. The fundamental measure theoretic result mentioned in the introduction is the following:

THEOREM 1. *Let $\eta_j(x)$ $(j = 1, 2, \ldots, n)$ be real non-negative $\mathcal{S}$-measurable functions satisfying*

$$\sum_{j=1}^{n} \eta_j(x) = 1 \qquad \text{for all } x \in X. \tag{1}$$

Then, if the set functions $\mu_k(S)$ $(k = 1, 2, \ldots, p)$ are non-atomic,[1] there exists a decomposition of X into n disjoint subsets $S_1, \ldots, S_n$ belonging to $\mathcal{S}$ which have the property that

$$\int_{S_j} \eta_j(x) d\mu_k(x) = \mu_k(S_j) \qquad (j = 1, 2, \ldots, n;\ k = 1, 2, \ldots, p). \tag{2}$$

The proof of this theorem makes use of a result of A. Liapounoff[2] on the ranges of vector measures and yields, incidentally, an extension of his result. The non-atomicity requirement is indispensable. It is this assumption that is responsible for the possibility to disregard mixed strategies in the games treated here, as opposed to the finite games originally considered by J. von Neumann.[3]

3. Consider now a two-person zero-sum game where player I has a finite number of pure strategies i $(i = 1, 2, \ldots, m)$ while the pure strategies of player II consist of $\mathcal{S}$-measurable functions $l(x)$ defined over X and

assuming only the values 1, 2, ..., n. The outcome of the game when the above pure strategies are adopted is given by

$$K(i, l(x)) = \sum_{j=1}^{n} \mu_{ij}(S_j), \tag{3}$$

where S_j is the set where $l(x)$ assumes the value j and the $\mu_{ij}(S)$ ($i = 1, \ldots, m$; $j = 1, \ldots, n$) are real-valued, bounded, countably additive set functions defined for $S \in \mathcal{S}$.

The general, mixed or pure, strategies of I may be represented by vectors $\xi = (\xi_1, \ldots, \xi_m)$ of non-negative components adding up to one, while the general strategy of II may be written as a vector function $\eta(x) = (\eta_1(x), \ldots, \eta_n(x))$ the components of which are non-negative S-measurable functions satisfying (1). When these strategies are adopted the outcome of the game is

$$K(\xi, \eta(x)) = \sum_{i=1}^{m} \xi_i \sum_{j=1}^{n} \int_X \eta_j(x) d\mu_{ij}(x).$$

A pure strategy of II may be also represented by a vector $\eta(x)$, only now the additional assumption is made that all components are either zero or one for all x. We easily deduce from Theorem 1 the following:

THEOREM 2. *If the set functions $\mu_{ij}(S)$ ($i = 1, \ldots, m$; $j = 1, \ldots, n$) are non-atomic, then to every strategy $\eta(x)$ of player II there exists a pure strategy $\eta^*(x)$ equivalent to it, i.e., such that $K(\xi, \eta(x)) = K(\xi, \eta^*(x))$ for all strategies ξ of player I.*

In most cases the functions $\mu_{ij}(S)$ are given by $\mu_{ij}(S) = \int_S a_{ij}(x) d\mu_i(x)$, where the $\mu_i(S)$ ($i = 1, \ldots, m$) have the properties described above and the $a_{ij}(x)$ ($j = 1, \ldots, n$) are $\mathcal{S}$-measurable real-valued functions integrable with respect to $\mu_i(S)$. The special case when the functions $a_{ij}(x)$ reduce to constants is particularly important. For this case we have

THEOREM 3. *If in the preceding theorem $\mu_{ij}(S) = a_{ij}\mu_i(S)$ then whatever $\eta(x)$ there exists a pure strategy $\eta^*(x)$ equivalent to it simultaneously for all choices of the constants a_{ij}.*

4. We now return to Theorem 1, but instead of considering the case where there existed a finite number of functions $\mu_k(S)$ we consider the case when k varies in some abstract space K. Let us introduce a metric g into the space K by putting $g(k, k') = \sup |\mu_k(S) - \mu_{k'}(S)|$, the sup being taken with respect to $S \in \mathcal{S}$. It is clear that if all other conditions of Theorem 1 are satisfied and the space M, with the metric g, is conditionally compact then, given $\epsilon > 0$, one may find a decomposition of X into disjoint $S_j \in \mathcal{S}$ such that

$$\left| \int_X \eta_j(x) d\mu_k(x) - \mu_k(S_j) \right| < \epsilon \qquad (j = 1, \ldots, n;\ k \in K).$$

We now consider games of the kind described in 3. except that we drop the restriction that i vary over a finite set and allow it to vary over an arbitrary space I. The outcome when pure strategies are adopted is again given by (3), while generally it is given by

$$K(\xi, \eta(x)) = \int_I \left\{ \sum_{j=1}^{n} \int_x \eta_j(x) d\mu_{ij}(x) \right\} d\xi_i,$$

$\xi = \xi_i$ being a probability distribution over I representing the first player's strategy.

From the preceding application of Theorem 1 we have:

THEOREM 4. *If the set functions $\mu_{ij}(S)$ $(i \in I, j = 1, \ldots, n)$ are non-atomic and I is conditionally compact according to the metric*

$$g(i, i') = \max_{j=1,\ldots,n} \sup_{S \in \mathcal{S}} |\mu_{ij}(S) - \mu_{i'j}(S)| \qquad (i, i' \in I)$$

then to every $\epsilon > 0$ and every strategy $\eta(x)$ of the second player, there exists a pure strategy $\eta^*(x)$ of this player satisfying $|K(\xi, \eta^*(x)) - K(\xi, \eta(x))| < \epsilon$ for all strategies ξ of the first player.

A theorem recently proved by R. Bellman and D. Blackwell[4] is a very special case of this result. Theorem 4 can evidently be extended to include the case when j also has an infinite range.

5. A rather extensive class of fixed sample size decision problems in statistics may be described as follows.[5] A random variable x with range in a t-dimensional Euclidean space X is distributed according to an unknown one of a finite number m of distributions, the possible (cumulative) distribution functions being $F_i(x)$ $(i = 1, \ldots, m)$. An observation (this means, in general, a sample of size t) is made and according to the observed value x the statistician may adopt any one of n decisions j. (In many cases $m = n$ and the statistician has to decide what is the true distribution.) If $F_i(x)$ is the actual distribution, x the observed value and the jth decision is adopted, then the statistician sustains a loss $w_{ij}(x)$, where $w_{ij}(x)$ is a finite real non-negative measurable function of x. If the statistician, on observing the value x, adopts the various decisions with probabilities $\eta_j(x)$ (these being non-negative measurable functions satisfying (1)), then the risk, or expected loss, when $F_i(x)$ is the true distribution function is

$$r_i(\eta) = \sum_{j=1}^{n} \int_x w_{ij}(x) \eta_j(x) dF_i(x).$$

We again say that the decision function $\eta(x)$ is non-randomized if for every x all but one of the $\eta_j(x)$ vanish.

There is no difficulty in deducing from Theorem 1 the following result.

THEOREM 5. *If the distribution functions are non-atomic[6] then given any decision function $\eta(x)$, there exists a non-randomized decision function $\eta^*(x)$ equivalent to it, i.e., such that $r_i(\eta^*) = r_i(\eta)$ for $i = 1, 2, \ldots, m$. If, more-*

over, the loss functions $w_{ij}(x)$ reduce to constants w_{ij}, there exists a non-randomized $\eta^*(x)$ equivalent to $\eta(x)$ simultaneously for all choices of the constants w_{ij}.

6. In a sequential procedure the sample size is not given in advance. In this case the problem may be presented as follows: An infinite sequence of real chance variables $(x_1, x_2, \ldots, x_t, \ldots)$ is distributed according to an unknown one of a finite number of distribution functions $F_i(x_1, x_2, \ldots, x_t, \ldots)$ $(i = 1, 2, \ldots, m)$.. The statistician has again a choice of a finite number n of (terminal) decisions. His decision rule δ consists of real, non-negative, measurable functions $\delta_{rt}(x_1, x_2, \ldots, x_t)$ $(r = 0, 1, \ldots, n; t = 1, 2, \ldots)$ satisfying

$$\sum_{r=0}^{n} \delta_{rt}(x_1, x_2, \ldots, x_t) = 1 \text{ for all } -\infty < x_1, \ldots, x_t < \infty.$$

The decision rule expressed by δ is interpreted thus: According to the observed value[7] x_1, the statistician decides either to continue experimentation and make another observation, or to stop further experimentation and adopt a terminal decision $j (j = 1, \ldots, n)$ with the respective probabilities $\delta_{01}(x_1)$ or $\delta_{j1}(x_1)$ $(j = 1, \ldots, n)$. If it is decided to continue experimentation, a value of x_2 is observed and it is again decided either to make a further observation or adopt a terminal decision j with respective probabilities $\delta_{02}(x_1, x_2)$ and $\delta_{j2}(x_1, x_2)$, etc., etc. The risk or expected loss if F_t is the true distribution and the decision rule δ is adopted is given by

$$r_t(\delta) = \sum_{t=1}^{\infty} \sum_{r=0}^{n} \int_{Y_t} v_{tr}(x_1, \ldots, x_t)\delta_{01}(x_1)\delta_{02}(x_1, x_2) \ldots \delta_{0(t-1)}(x_1 \ldots, x_{t-1}) \cdot \delta_{rt}(x_1, \ldots, x_t) dF_{tt}(x_1, \ldots, x_t),$$

where Y_t is the t dimensional space of $x_1, \ldots, x_t$, $v_{tr}(x_1, \ldots, x_t)$ are real finite non-negative measurable functions (representing total loss and cost of experimentation) and $F_{tt}(x_1, \ldots, x_t)$ is the joint distribution function of $x_1, \ldots, x_t$ when F_t is the true distribution. The sequential decision rule δ is called non-randomized if for all $x_1, \ldots, x_t$ $(t = 1, 2, \ldots)$ all but one of the $n + 1$ function $\delta_{01}(x_1, \ldots, x_t), \ldots, \delta_{nt}(x_1, \ldots, x_t)$ are equal to zero. Here we have the result:

THEOREM 6. *If the m one-dimensional distribution functions $F_i(x_1)$ $(i = 1, \ldots, m)$ are continuous, then given any sequential decision rule, there exists a non-randomized sequential decision rule equivalent to it. If, moreover, the functions $v_{tr}(x_1, \ldots, x_t)$ reduce to constants v_{tr}, there exists a non-randomized decision function, equivalent to the given one, simultaneously for all choices of the constants v_{tr}.*

In statistical applications $v_{0t}(x_1, \ldots, x_t)$ is usually zero, and one considers only decision rules for which the probability is one of terminating

experimentation in a finite number of steps. Theorem 6 remains valid if these restrictions are explicitly made.

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[1] I.e., if for some $S \in \mathfrak{S}$ and $1 \leq k \leq \rho$, $\mu_k(S) \neq 0$, then there exists an $S' \subset S$ also belonging to $\mathfrak{S}$ and such that $\mu_k(S')$ is neither zero nor equal to $\mu_k(S)$.

[2] *Bull. Acad. Sci. U.R.S.S.*, **4** 465–478 (1940). Cf. also Halmos, P. R., *Bull. Am. Math. Soc.*, **54** 416–421 (1948). The essential part of Liapounoff's theorem was again proved independently by Neyman, J., *C.R. Acad. Sci. Paris*, **222** 843–845 (1946).

[3] Cf., e.g., Neumann, J. v., and Morgenstern, O., *The Theory of Games and Economic Behavior*, 2nd ed., Princeton, 1947.

[4] These PROCEEDINGS, **35** 600–605 (1949).

[5] Cf., e.g., Wald, A., *Ann. Math. Stat.*, **20** 165–205 (1949).

[6] This means, in the present case, that no single point is assigned positive probability.

[7] We exclude those procedures in which there is a positive probability of reaching a final decision before making even a single observation.

ON MULTIPLICATIVE PROPERTIES OF A GENERALIZED JACOBI-CAUCHY CYCLOTOMIC SUM

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In this article we shall consider the exponential sum,[1] for $s > 1$,

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \sum_{a_1, \cdots, a_{s-1}} \alpha_s^{\mu_s \operatorname{ind} A} \prod_{i=1}^{s-1} \alpha_i^{\mu_i \operatorname{ind} a_i}; \qquad (1)$$

$$\psi(\alpha_i^{\mu_i}) = 1, \qquad (1a)$$

where $\alpha_i = e^{2\pi i/m_i}$; $i = 1, 2, \ldots, s$; $p^n - 1 \equiv 0 \pmod{m_i}$, p is an odd prime; μ_i, $i = 1, 2, \ldots, s$ integers ≥ 0; $\operatorname{ind} a_i$ is defined by $g^{\operatorname{ind} a_i} = a_i$, in a finite field of order p^n, designated by $F(p^n)$, or K; g denotes a generator of the cyclic group formed by the non-zero elements of $F(p^n)$ under multiplication, $\alpha_i^{\mu_i \operatorname{ind}(0)} = 0$ for any μ_i, and each a_i ranges independently over each element of K, with $A = 1 - \sum_{i=1}^{s-1} a_i$.

In the article just cited (relation (17)) a relation was found for the number of solutions in $\gamma_1, \gamma_2, \ldots, \gamma_s$ of

$$1 + \sum_{i=1}^{s} g^{j_i + m_i \gamma_i} = 0 \qquad (2)$$

where γ_i is in the range 0, 1, ..., $(m_i' - 1)$, with $m_i m_i' = p^n - 1$. This result may be regarded as an additive property of the number ψ. Here we shall be more interested in multiplicative properties of it, particularly its decomposition into factors in various ways. The sum ψ is an algebraic integer in the field $k(\alpha)$ with $\alpha = e^{2\pi i/m}$ and $m = p^n - 1$, and from an arithmetic standpoint questions of factorization are often fundamental. The arguments we shall use are, in the main, extensions of known methods for deriving properties of (1) when $s = 2$. In spite of the fact that our ψ seems to be quite a complicated number, relations (15), (17) and (20) turned out to be much simpler than what we originally expected them to be.

We also shall consider

$$\tau(\alpha^\mu) = \sum_{a \bmod K} \alpha^{\mu \operatorname{ind} a} \zeta^{\operatorname{tr}(a)}, \tag{3}$$

with μ any integer and we define tr(a) as follows: It is known that $a + a^{(1)} + \ldots + a^{(n-1)} = c$ where c is an element of $F(p)$, and $(k) = \mathfrak{p}^k$. If we take the residue classes modulo p which form a field isomorphic to $F(p)$ and c maps on a residue class defined by the integer d, then we set $\zeta^{\operatorname{tr}(a)} = \zeta^d$. From the definitions of tr (a) and ind a it follows easily that

$$\operatorname{ind} a + \operatorname{ind} b \equiv \operatorname{ind}(ab) \pmod{(p^n - 1)}, \quad \operatorname{tr}(a) + \operatorname{tr}(b) = \operatorname{tr}(a + b).$$

The following relations are known:

$$\sum_{\substack{a \bmod K \\ a \neq 0}} \zeta^{\operatorname{tr}(ac)} = \begin{cases} -1 \text{ if } c \not\equiv 0, \\ p^n - 1 \text{ if } c = 0; \end{cases} \tag{4}$$

$$\sum_{d=0}^{vm_i - 1} \alpha_i^{cd} = \begin{cases} 0;\ c \not\equiv 0 \pmod{m_i}, \\ vm_i;\ c \equiv 0 \pmod{m_i}; \end{cases} \tag{5}$$

as well as an important property of (3), namely

$$\tau(\alpha_i^{\mu_i})\tau(\alpha_i^{-\mu_i}) = \alpha_i^{\mu_i \operatorname{ind}(-1)} p^n, \tag{6}$$

where $\alpha_i^{\mu_i} \neq 1$. This relation will be generalized in (9).

In order to prove two theorems concerning the factoring of $\psi(\alpha_1^{\mu_1}, \alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s})$ we shall first consider

$$\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i}) = \sum_{a_1, \ldots, a_s} \prod_{i=1}^{s} \alpha_i^{\mu_i \operatorname{ind} a_i} \zeta^{\operatorname{tr}(a_1 + \cdots + a_s)} \tag{7}$$

Under the assumption that $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$, this may be reduced by letting $a_i = -b_i a_1$, $i \geq 2$; (7) becomes,

$$\sum_{a_1, b_2, \ldots, b_s} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\operatorname{ind} a_1} \prod_{i=2}^{s} \alpha_i^{\mu_i \operatorname{ind} b_i} \cdot \left(\prod_{i=2}^{s} \alpha_i^{\mu_i} \right)^{\operatorname{ind}(-1)} \zeta^{\operatorname{tr} a_1(1 - b_2 - \cdots - b_s)}.$$

Since $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$ and $\alpha_1^{-\mu_1} = \prod_{i=2}^{s} \alpha_i^{\mu_i}$ the above may be written

$$\sum_{b_2, \cdots, b_s} \prod_{i=1}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)} \cdot \sum_{a_1} \zeta^{\text{tr } a_1(1 - b_2 - \cdots - b_s)}.$$

Using (4) then (7) reduces, when we carry out the second summation, to

$$-\sum \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)} + (p^n - 1) \sum \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)}, \quad (8)$$

where in the first summation each b ranges over all elements of $F(p^n)$ such that $1 - b_2 - \ldots - b_s \neq 0$ and in the second, over all elements of $F(p^n)$ where such is not the case.

For $s = 2$, the expression (8) reduces to $p^n \alpha_1^{-\mu_1 \text{ ind } (-1)}$.

For $s > 2$, recombining the terms in (8) gives, for the right-hand member of (7),

$$p^n \sum_{b_2, \cdots, b_{s-1}} \prod_{i=2}^{s-1} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_s^{\mu_s \text{ ind } (1 - b_2 \cdots b_{s-1})} \cdot \alpha_1^{-\mu_1 \text{ ind } (-1)} - \sum_{b_2, \cdots, b_s} \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)}.$$

The second term is zero unless $\mu_i \equiv 0 \ (m_i)$ for all i; therefore, we may now write,

$$\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i}) = p^n \psi(\alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s}) \alpha_1^{\mu_1 \text{ ind } (-1)} - \begin{cases} 0, \text{ if } \mu_i \not\equiv 0 (m_i) \text{ for some } i > 1, \\ (p^n - 1)^{s-1}, \text{ if } \mu_i \equiv 0 (m_i) \text{ for each } i; \end{cases} \quad (9)$$

where $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$. For $s = 2$ we obtain (6), using (1a). If we employ the relation,

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = -\psi(\alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s}) \alpha_1^{-\mu_1 \text{ ind } (-1)}$$

where $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$, and $\mu_i \not\equiv 0 \ (m_i)$ for any i (the proof of this result will appear in a paper to be published later by one of the authors), we then have[2] by (9),

LEMMA I: *If* $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$ and $\mu_i \not\equiv 0 \ (m_i)$ for any i then

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \frac{\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i})}{-p^n}. \quad (10)$$

Now we consider (7) under the assumption that $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$. Let $a_1 + \ldots + a_s = c$. Consider first the case where $c = 0$; if we let $a_i = b_i a$, where $i \geq 2$ then since $1 + b_2 + \ldots + b_s = 0$ we may write (7), on the right, under the above assumption,

$$\sum_{a_1, b_2, \cdots, b_{s-1}} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } a_1} \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \cdot \alpha_s^{\mu_s \text{ ind } (-1 - b_2 - \cdots - b_{s-1})}.$$

Since $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$, $\sum_{a_1} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } a_1} = 0$ thus in the case where $c = 0$, (7) equals zero. Consider now the case where $c \neq 0$. If we let $a_i = c\, b_i$, then since $b_1 + b_2 + \ldots + b_s = 1$, we may under these conditions write the right-hand member of (7) as

$$\sum_{b_1, \cdots, b_{s-1}} \prod_{i=1}^{s-1} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_s^{\mu_s \text{ ind } (1 - b_1 - \cdots - b_{s-1})} \cdot \sum_{c} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } c} \zeta^{\text{tr } c},$$

which in view of (1) and (3) may be written,

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) \cdot \tau\left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right).$$

Thus we now have

LEMMA II: *If* $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$, *then*

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \frac{\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i})}{\tau\left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)}. \tag{11}$$

From this we immediately obtain[3]

$$\psi\bar{\psi} = p^{n(s-1)} \tag{12a}$$

provided $\alpha_i^{\mu_i} \neq 1$, $i = 1, \ldots s$; $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$; on using (6).

Set, if $s > t$,

$$\psi_{t,s} = \psi\left(\prod_{i=1}^{t} \alpha_i^{\mu_i}, \alpha_{t+1}^{\mu_{t+1}}, \ldots, \alpha_s^{\mu_s} \right). \tag{12}$$

We now proceed to factorize ψ, distinguishing four cases, I–IV. Throughout the discussion of the four cases we assume $s > t > 1$ and $\mu_i \not\equiv 0 \pmod{m_1}$ for each i.

I. If $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$ then by (10), and using the notation indicated in (12), we have

$$\psi_{1,s} = \frac{\prod_{i=1}^{t} \tau(\alpha_i^{m_i}) \cdot \prod_{i=t+1}^{s} \tau(\alpha_i^{m_i})}{-p^n} \tag{13}$$

Suppose $\prod_{i=1}^{t} \alpha_i^{m_i} = 1$. If $t = s - 1$, then $\mu_s \equiv 0 \ (m_s)$, which is contrary to the original assumption; thus we may assume $t < s - 1$ noting that $s > 2$ by hypothesis. The right hand member of (13) then becomes by (10), noting that $\alpha_{t+1}^{m_{t+1}} \cdots \alpha_s^{m_s} = 1$,

$$\psi_{1,s} = -p^n \psi_{1,t} \cdot \psi(\alpha_{t+1}^{m_{t+1}}, \ldots, \alpha_s^{m_s}). \tag{13a}$$

II. Suppose $\prod_{i=1}^{t} \alpha_i^{m_i} \not\equiv 1$, with $\prod_{i=1}^{s} \alpha_i^{m_i} = 1$. If $t < s$, (13) may be written as

$$\frac{\prod_{i=1}^{t} \tau(\alpha_i^{m_i})}{\tau\left(\prod_{i=1}^{t} \alpha_i^{m_i}\right)} \cdot \frac{\tau\left(\prod_{i=1}^{t} \alpha_i^{m_i}\right) \prod_{i=t+1}^{s} \tau(\alpha_i^{m_i})}{-p^n},$$

which becomes by (10) and (11)

$$\psi_{1,t}\psi_{1,s} = \psi_{1,s}. \tag{13b}$$

III. If $\prod_{i=1}^{s} \alpha_i^{m_i} \not\equiv 1$ with $\prod_{i=1}^{t} \alpha_i^{m_i} = 1$ then by (10) we have

$$\psi_{1,s} = \frac{\prod_{i=1}^{t} \tau(\alpha_i^{m_i}) \prod_{i=t+1}^{s} \tau(\alpha_i^{m_i})}{\tau\left(\prod_{i=1}^{s} \alpha_i^{m_i}\right)}. \tag{14}$$

Since $\tau\left(\prod_{i=1}^{s} \alpha_i^{m_i}\right) = \tau\left(\prod_{i=t+1}^{s} \alpha_i^{m_i}\right)$, (14) may be written as

$$-p^n \frac{\prod_{i=1}^{t} \tau(\alpha_i^{m_i})}{-p^n} \frac{\prod_{i=t+1}^{s} \tau(\alpha_i^{m_i})}{\tau\left(\prod_{i=t+1}^{s} \alpha_i^{m_i}\right)},$$

which becomes by (10) and (11)

$$\psi_{1,s} = -p^n \psi_{1,t} \psi(\alpha^{m_{t+1}}_{t+1}, \ldots, \alpha_s^{m_s}). \tag{14a}$$

IV. Suppose $\prod_{i=1}^{t} \alpha_i^{m_i} \not\equiv 1$ with $\prod_{i=1}^{s} \alpha_i^{m_i} \not\equiv 1$; (14) may be written

$$\frac{\prod_{i=1}^{t}\tau(\alpha_i^{\mu_i})}{\tau\left(\prod_{i=1}^{t}\alpha_i^{\mu_i}\right)}\,\frac{\tau\left(\prod_{i=1}^{t}\alpha_i^{\mu_i}\right)\prod_{i=t+1}^{s}\tau(\alpha_i^{\mu_i})}{\tau\left(\prod_{i=1}^{s}\alpha_i^{\mu_i}\right)},$$

which becomes by (11)

$$\psi_{1,t}\psi_{t,s} = \psi_{1,s}. \tag{14b}$$

Thus we have, since results (13*a*), (13*b*) and (14*a*), (14*b*) are the same, respectively:

THEOREM I: *If* $\mu_t \not\equiv 0(m_t)$ *and* $s > t > 1$, *then*

$$\psi_{1,s} = \begin{cases} \psi_{1,t}\psi_{t,s}, \text{ if } \prod_{i=1}^{t}\alpha_i^{\mu_i} \not\equiv 1; \\ -p^n\psi_{1,t}\psi\,(\alpha_{t+1}^{\mu_{t+1}}, \ldots, \alpha_s^{\mu_s}) \text{ if } \prod_{i=1}^{t}\alpha_i^{\mu_i} = 1, \end{cases} \tag{15}$$

the symbols used being defined in (1) *and* (12).

Suppose $t_1 > t_2 > \ldots > t_k$, $s > t_i \geq 2$, are the distinct values of t for which $\prod_{i=1}^{t}\alpha_i^{\mu_i} = 1$ and assume also that $\mu_t \not\equiv 0(m_t)$. By Theorem I we may write

$$\psi_{1,s} = \psi_{1,t+1}\psi_{t+1,s}. \tag{16}$$

By continually reapplying said Theorem, (16) becomes, since t_1 exceeds any other t,

$$\psi_{1,s} = -p^n\psi_{1,t_1}\psi_{t_1+1,\,t_1+2}\cdots\psi_{s-1,\,s}.$$

Repeated application of this relation and (15) gives

$$\psi_{1,s} = (-p^n)^k\frac{\psi_{1,2}\psi_{2,3}\cdots\psi_{s-1,\,s}}{\psi_{t_1,\,t_1+1}\cdots\psi_{t_k,\,t_k+1}},$$

but $\psi_{t_i,\,t_i+1} = \sum_a (1)^{\text{ind } a}(\alpha_{t+1}^{\mu_{t+1}})^{\text{ind }(1-a)}$, and since $\alpha_{t_i+1}^{\mu_{t_i+1}} \not\equiv 1$, $\psi_{t_i,\,t_i+1} = -1$, then we have[4]

THEOREM II: *If* $\mu_t \not\equiv 0(m_t), i = 1, 2, \ldots, s$, *and* k *represents the number of distinct* t*'s for which* $\prod_{i=1}^{t}\alpha_i^{\mu_i} = 1$ *where* $t < s$, *then*

$$\psi_{1,s} = p^{kn}\psi_{1,2}\psi_{2,3}\cdots\psi_{s-1,\,s}, \tag{17}$$

the symbols used being defined in (1) *and* (12).

The condition, $\mu_t \not\equiv 0(m_t)$, does not constitute a restriction on the generality of this result since we can reduce a ψ number for which $\mu_i \equiv 0(m_t)$ for some i's to a ψ number involving only those $\alpha_i^{\mu_i}$ for which $\mu_t \not\equiv 0(m_t)$.

For the case where $m_1, m_2, \ldots, m_s$ are prime each to each then $\prod_{i=1}^{t} \alpha_i^{\mu_i} = 1$ if and only if $\mu_i \equiv 0(m_i)$, $i = 1, 2, \ldots, t$. Hence we have the

COROLLARY. *If $m_1, m_2, \ldots, m_s$, defined in connection with (1), are prime each to each, then*

$$\psi_{1,s} = \psi_{1,2}\psi_{2,3}\ldots\psi_{s-1,s} \tag{18}$$

From Theorem II we obtain in general a great variety of decompositions of ψ by renumbering the quantities

$$\alpha_1^{\mu_1}, \alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s},$$

as, say,

$$\alpha_{h_1}^{\mu_{h_1}}, \alpha_{h_2}^{\mu_{h_2}}, \ldots, \alpha_{h_s}^{\mu_{h_s}},$$

the latter being any permutation of the original set, and letting $\alpha_i^{\mu_i}$ be replaced by $\alpha_{h_i}^{\mu_{h_i}}$ in the proof of (17); $i = 1, 2, \ldots, s$.

The form of (17) enables us to obtain a number of multiplicative results concerning $\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s})$ from the multiplicative properties of $\psi(\alpha_h^{\mu_h}, \alpha_k^{\mu_k})$. For example let m be the least common multiple of $m_1, m_2, \ldots, m_s$ then any $\psi_{h,h+1}$ may be written in the form

$$\psi_{h,h+1} = \sum_{a \in K} \beta^{b \text{ ind } a + d \text{ ind } (1-a)} \tag{19}$$

where $\beta = e^{2i\pi/m}$ and b and d are some integers. Suppose we define a number $\psi_{h,h+1}^{(r)}$ by using $F(p^{rn})$ in place of $F(p^n)$ in (19), then further assume that $g_r^{c_r} = g^c$ where g_r is a multiplicative generator of the cyclic group formed by the non-zero elements of $F(p^{rn})$, g has the same property for $F(p^n)$, and also

$$c_r = \frac{p^{rn} - 1}{m}, \qquad c = \frac{p^n - 1}{m}.$$

Then H. H. Mitchell[5] showed that

$$\psi_{h,h+1}^{(r)} = (-1)^{r-1}(\psi_{h,h+1})^r$$

unless both b and d are $\equiv 0 (\text{mod } m)$. Using this together with (17) gives immediately

$$(\psi_{1,s})^r = (-1)^{(s-1)(r-1)} p^{(r-1)kn} \psi_{1,s}^{(r)} \tag{20}$$

for $\mu_i \not\equiv 0 \pmod{m_i}$, $i = 1, 2, \ldots, s$, and k is defined as in Theorem II.

[1] Vandiver, these PROCEEDINGS, **36**, 144 (1950), with references there given.

[2] Weil, *Bull. Am. Math. Soc.*, **55**, 501 (1949), last relation on said page, and relation (13) of the reference given in our first footnote. Our relation (9) of the present paper, not given by Weil, is more convenient than (10) for some purposes.

[3] Weil, *loc. cit.*, p. 501, gave an argument different at least in form to derive a corresponding property to this for his number $j(\alpha)$ which is a special case of our number ψ of (1).

[4] For $n = 1$, the nearest approach to the process used here seems to be found in H. J. S. Smith's Report on the Theory of Numbers, Collected Works, Vol. I, 271–272, Oxford (1894); reproduced by P. Bachmann, *Die Lehre von der Kreisteilung*, 279–281, Leipzig and Berlin (1921). However, our number ψ of (1) for $n = 1$ is not exhibited explicitly by them in terms of the α's alone, but is defined by means of the $\tau(\alpha^n)$ of our relation (3).

[5] *Ann. Math.*, **18**, 120 (1917).

THE SOLUTION OF A CERTAIN GENERAL TYPE OF INTEGRAL EQUATION

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The following integral equation arose in an engineering design problem:

$$\int_0^1 [f''(1)f(u) + 2f'^2(u)]du = 0. \tag{1}$$

The only restriction placed on the function $f(u)$ is that it be analytic.

If we assume that $f(u)$ has the form

$$f(u) = F(u) + Au, \tag{2}$$

where $F(u)$ is any given analytic function, then equation (1) takes the form

$$\int_0^1 [F''(1)F(u) + AuF''(1) + 2F'^2(u) + 2A^2 + 4AF'(u)]du = 0 \tag{3}$$

or, on performing the integrations

$$2A^2 + 4A[F(1) - F(0) + {}^1/_8F''(1)] + \int_0^1 [F''(1)F(u) + 2F'^2(u)]du = 0. \tag{4}$$

This quadratic equation in A has two solutions for every given $F(u)$.

We have thus found the expression for A which makes equation (2) become a solution of equation (1).

It is obvious that a solution of Type (2) exists for a much more general type of integral equation than (1). Consider a function ϕ which is rational and analytic in $f(u)$ and its various derivatives but does not involve the independent variable u explicitly. The integral equation

$$\int_0^1 \phi[f(u), f'(u), f''(u), \ldots]du = 0 \tag{5}$$

will have a solution of Type (2), since by substituting equation (2) and its derivatives for $f(u)$ and its derivatives and performing the integrations, we obtain an algebraic equation for A which can be solved at least in principle.

It should be possible to extend these results to still more general types of integral equations.

EXTRACTION OF A MATING REACTION INHIBITING AGENT FROM PARAMECIUM CALKINSI*

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Since interaction of mating-type substances at the surfaces of conjugating paramecia may initiate the physiological changes of fertilization in these animals,[1] a detailed study of the nature and manner of interaction of these substances is warranted. So far all attempts to obtain an active extract of mating substance from paramecia have failed.[2, 3] Furthermore all mating reactivity disappears when these animals are thoroughly broken up. This apparent disappearance of mating substance activity from both extract and residue suggests the release of some mating substance inhibiting agent. Such an agent has now been obtained from homogenized *Paramecium calkinsi*. The presence of this agent can account for disappearance of mating substance activity in certain, but not in all, extraction procedures.

Preparation of the Agent.—*Paramecium calkinsi* were grown in a 2/5 sea water-baked lettuce infusion which had previously been inoculated with *Aerobacter aerogenes*. To prepare paramecium extracts the animals in four to six liters of paramecium culture were concentrated to 25–50 ml. by passing the culture through a porous-grade Berkefeld filter (the senior author's method has been described fully by Sonneborn).[4] The animals were further concentrated by centrifugation, washed in 2/5 sea water and taken up in one to three ml. of 2/5 sea water. These concentrated animals were then homogenized or were lyophilized[5] and later taken up in distilled water and homogenized. The crude homogenate was then centrifuged or passed through a sintered glass filter to give an opalescent supernatant or filtrate.

The most practical method for detecting any mating-type substance inactivating agent is to treat reactive dead paramecia with the agent, wash the dead animals free of the agent and then test them for specific mating reactivity with living animals. When testing paramecium homogenates for mating substance inactivating action the use of reactive dead animals in preference to living animals is essential because living animals may be killed by a non-mating-type specific heat labile auto-toxin frequently present in homogenates (Metz, unpublished). This introduces a complicating factor which cannot be controlled. If living animals are not killed by the homogenate, they can feed upon it with consequent loss of mating reactivity due to overfeeding.

The dead reactive paramecia used in these experiments were prepared

by treating cultures of *P. calkinsi* with formalin or picric acid (19 or 15 volumes of culture to one volume of formalin or saturated picric acid solution, respectively) for one hour. After the treatment the animals were washed in 2/5 sea water and suspended in this saline. Properly selected cultures of reactive Type I *P. calkinsi* that have been killed in this manner give very strong mating reactions with living Type II animals. These dead animals will even induce meiosis, macronuclear breakdown and pseudoselfing pair formation in living Type II animals.[3] Formalin-killed Type II animals at best give only moderately strong reactions with living Type I animals, whereas picric acid-killed Type II animals can give very strong reactions.

To test for inhibiting action one to several volumes of the opalescent extracts were mixed with a given volume of strongly reactive dead paramecia (usually two drops containing roughly 1000 dead animals). After an exposure of one to two hours the extract was withdrawn from the treated dead animals. These were then washed in 2/5 sea water and tested for mating reactivity by mixing with living reactive animals of the same and opposite mating type. Generally such extract-treated dead animals failed to give mating reactions or at best gave only weak transitory reactions as compared to control dead animals buffered at the pH (5.5) of the extract.

Relation of the Inhibiting Agent to the Mating Substances.—Apparently the mating substance inhibiting agent of the extracts is not the mating substance of the extracted animals. This follows from the fact that the inhibiting agent is non-specific. Thus the extract prepared from mating Type I *P. calkinsi* inhibited dead reactive animals of both Type I and Type II. Moreover *P. calkinsi* I extract inhibited formalin-killed reactive variety 4 (Types VII and VIII) *Paramecium aurelia*. Extracts prepared from Type II *P. calkinsi* were also non-specific in action. If the inhibiting agent were the mating substance of the extracted animals, it should show mating type specificity. It would be expected to react with and inhibit only animals of the complementary mating type, not animals of the mating type from which the extract was prepared.

In view of the non-specific character of the inhibiting effect, one system, namely the action of Type I extract on Type I animals, was selected for further study.

Mode of Action of the Agent.—The inhibiting action of homogenates was not altered by repeated centrifugation or by passage through a sintered glass filter. Thus the inhibiting action cannot be attributed to a simple mechanical factor such as clogging of the cilia of the treated dead animals with debris. Therefore it is concluded that inhibition results from chemical combination of the mating substance with some agent in the extracts.

Apparently the non-specific mating substance inhibiting agent does not

act by combining with the mating substance of dead animals in antigen-antibody-like fashion. This follows from the fact that all attempts to absorb the inhibiting agent of Type I extracts by living or dead Type I paramecia have failed, and suggests that the agent may inactivate the mating substance by enzymatic action. Furthermore the agent acts slowly and only in low dilution. This is indicated by the experiment presented in table 1. The extract used in this experiment was prepared by concentrating six liters of culture (0.8 cc. packed paramecia) and homogenizing the animals in 3 cc. of 2/5 sea water after lyophilization.

Unfortunately there is no convenient objective assay method available for determining the amount of active mating substance present on dead or living paramecia. However, the relative degree of reactivity as indicated subjectively in table 1 shows that little if any inactivation occurred during the first 20 minutes of exposure to extract even in the highest extract concentration. Sixty minutes' exposure to the extract were required for

TABLE 1

EFFECT OF EXTRACT CONCENTRATION ON RATE OF INHIBITION OF FORMALIN-KILLED PARAMECIUM CALKINSI TYPE I BY P. CALKINSI TYPE I EXTRACT

TIME:	20 MIN.	40 MIN.	60 MIN.
Extract dilution			
5/7	++++	+	±
2.5/7	++++	+++	++
1.25/7	++++	++++	+++
0.65/7	++++	++++	++++
pH 5.5 buffer	++++	++++	++++

nearly complete inactivation of the formalin-killed Type I animals. The reactivity of the test animals in the fourfold (0.65/7) extract dilution was not significantly different from that of controls in buffer solution even after a 60-minute treatment with the extract.

It is apparent from this experiment that the inhibiting agent acts slowly even at the lowest dilutions used and that the rate of inactivation is a function of the concentration of the agent.

Properties of the Agent.—Preliminary attempts to characterize the inhibiting agent of Type I animals show that it is heat labile to the extent that its action is rapidly destroyed at 100°C. It is non-dialyzable and evidently stable in absolute acetone since paramecia which have been lyophilized, treated with absolute acetone, dried and finally extracted yield active preparations of the inhibiting agent. Extracts of formalin-killed Type I animals, however, do not inhibit Type I or Type II animals. Attempts to salt out the agent with ammonium sulfate have failed. The $(NH_4)_2SO_4$ precipitates and supernatants were inactive both separately and when combined. Probably the agent was inactivated in the process of removing the salt.

These fragmentary data indicate that the inhibiting agent is a rather labile substance of fairly high molecular weight and suggest that it may be a protein. This is in accord with the view that its action is enzymatic.

Discussion.—As mentioned previously, the mating substance inhibiting agent cannot be the mating substance of the extracted animals. This follows from the non-specific character of its action, from the fact that it cannot be extracted from formalin-killed reactive *Paramecium calkinsi* and that it can be obtained from animals which are not in mating condition. The presence of this agent can account for failure to obtain active extracts of mating substance from homogenized living or lyophilized animals. However, it cannot account for disappearance of mating substance activity in both extract and residue of homogenized animals that have received sufficiently harsh treatment (i.e., formalin) to destroy the inhibiting agent without destroying the mating substance. Disappearance of mating substance activity under these conditions cannot be explained readily except by assuming that essential structural relations of the cell surface are destroyed by the mechanical extraction procedure used. At present there is no evidence that any such gross steric factors are essential for mating substance activity.

This study suggests that the mating substance inhibiting agent is enzymatic in action. If the agent should prove to be an enzyme and if the chemical nature of its action could be demonstrated with a well-defined artificial substrate, knowledge of the nature of the mating substance might be considered advanced. Such an enzyme might be concerned specifically with the characteristic appearance and disappearance of mating reactivity in normal living animals in accordance with their nutritional state. It is also possible that the agent is in the nature of a "digestive" enzyme which bears no physiological relation to the mating substance. Either of these possibilities is of sufficient interest to warrant further investigation of the mating substance inactivating agent.

Summary.—Extracts of *Paramecium calkinsi* which inhibit the mating reactivity of formalin or picric acid-killed Paramecium are described. The extracts are not species or mating type specific in action. Therefore it is concluded that the active agent in the extracts is not the mating substance of the extracted animals. Since the inhibiting agent is not absorbed by paramecia it is suggested that it may be an enzyme. The possible relation of the inhibiting agent to the mating substance is discussed.

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[5] Metz, C. B., and Fusco, E. M., *Biol. Bull.*, **97**, 245 (1949).

ON THE RATE OF PASSIVE SINKING OF DAPHNIA

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The resistance encountered by a body falling in a viscous liquid is given approximately by dimensional analysis as

$$R = kd^q v^q \rho^{q-1} \mu^{2-q} \tag{1}$$

where d is an appropriate linear dimension, v the velocity of the body, ρ the density of the medium and μ the viscosity of the medium. When the resistance exactly balances the force of gravity the velocity becomes constant and is given by

$$v^q = Kd^{3-q}(\rho' - \rho)\rho^{1-q}\mu^{q-2}, \tag{2}$$

where K is constant for bodies of the same shape and ρ' is the density of such bodies.

For small bodies falling slowly in media of high viscosity, the resistance will be determined by the viscosity of the medium but not by the density. In this case $q = 1$ and

$$v \propto d^2(\rho' - \rho)\mu^{-1}. \tag{3}$$

This is the general form of the relationship deduced just a century ago by Stokes for a small sphere falling slowly.

For large bodies falling so rapidly that viscosity is of no consequence in comparison with the inertia of the liquid, $q = 2$ and

$$v \propto \sqrt{d(\rho' - \rho)\rho^{-1}}. \tag{4}$$

This is the general form of the relationship deduced by Newton late in the 17th century, and which holds with fair accuracy for some cases of large rapidly falling bodies.[1]

In general Stokes' law corresponds to laminar flow and Newton's law to turbulent flow of the liquid past the body. It is generally conceded that Stokes' law holds with considerable accuracy when Reynolds' Number, N, <0.5, where

$$N = \frac{dv\rho}{\mu}. \tag{5}$$

Newton's law, however, does not apply immediately when N exceeds 0.5, and over a certain fairly wide interval there is empirical evidence that $q = \frac{3}{2}$,[1] and therefore

$$v \propto d_0 \sqrt[3]{(\rho' - \rho)^{2-1-1}_{\rho\mu}}. \tag{6}$$

In this expression the linear dimension d_0 is given by

$$d_0 = d - \xi d_s \tag{7}$$

where d_s, the so-called critical diameter, is given, according to Allen, by

$$d_s = \sqrt[3]{\frac{9\mu^2}{2g\rho(\rho' - \rho)}} \tag{8}$$

and ξ is a constant depending on the shape of the falling body.

Little data exists on the rate of passive sinking of freshwater planktonic organisms. Such information as has been recorded suggests that in the cases of bacteria, protozoa, unicellular algae and nearly all rotifers $N \leq 0.5$ and the rate of falling is therefore likely to be directly proportional to the square of the appropriate linear diameter, and to the density difference, and inversely proportional to the viscosity. For somewhat larger organisms the less well-known relationship derived by Allen may be expected to hold, the velocity being directly proportional to the appropriate linear diameter less a small correction, to the two-thirds power of the density difference and inversely to the cube root of the viscosity and density of the medium. It is unlikely that Newton's law is of any interest in the study of the plankton. Bowkiewicz[2] who alone has attempted to go beyond Stokes' law in the study of sinking speeds unfortunately made two mistakes, one in the interpretation of his data and one in supposing that the reciprocal of the square root of the viscosity entered into Newton's formulation.

We have determined the rate of falling of a number of specimens of *Daphnia* sp. 1[3] of various sizes from Bantam Lake, Conn., at temperatures between 21.7° and 27.2°C., corresponding to viscosities between 0.0085 and 0.0097 poise. All specimens were narcotized with ethyl urethane and had open antennae; they sank at various angles, the long axis being inclined with the head upward at angles from about 10° to about 80° above the horizon. The experiments were conducted in a cylinder of internal diameter 7.8 cms. For a sphere of diameter d falling in such a cylinder the velocity must be raised by a factor of $\left(1 + \frac{2.1d}{7.8}\right)$ according to Ladenburg's correction for the effect of the walls of the vessel. In the case of our smallest animal if d be taken as the length of the body the correction would amount to multiplication by 1.016, in the case of the largest to multiplication by 1.048. Actually a sphere of diameter d has a larger volume than an animal of length d and it is practically certain that such a correction would be excessive.

In figure 1 all the data, uncorrected for the effect of the walls, have been plotted against body length (exclusive of tail spine) on a double logarithmic grid. The maximum effect of the size of the vessel is indicated by the single open circle which is the corrected position of the point immediately below it, and which almost certainly represents an overcorrection. Although there is a fair amount of irregularity, it is evident that the points tend to fall along the line of slope 2, corresponding to direct proportionality between velocity and the square of the length. It is probable that most of the irregularity is due to differences in the density dependent on differences

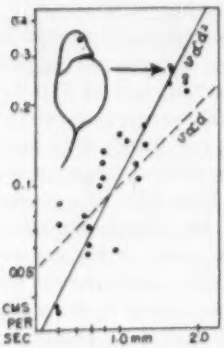

FIGURE 1

Relation of sinking velocity of *Daphnia* sp. 1 to length (tail spine excluded), the open circle indicates the maximum effect of Ladenburg's correction. Inset shows an experimental animal in lateral view.

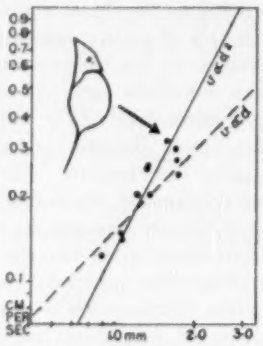

FIGURE 2

Relation of sinking velocity of *Daphnia* sp. 2 to length (tail spine excluded). Inset of an experimental animal in lateral view.

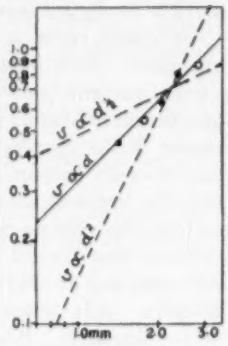

FIGURE 3

Relation of sinking velocity of *Daphnia pulex* to length, from the data of Eyden;[4] solid circles measured individuals, open circles means of groups.

in the nutritive condition of the animal. In so far as the rather uncertain correction for the effect of the walls of the vessel is significant, it would raise the values of the velocity for the larger animals a little, improving the fit at the upper end of the curve to a very slight extent.

A few determinations of sinking speeds for a series of animals of almost identical lengths (0.86–0.89 mm.) were made at different temperatures and so at different viscosities. The results are plotted in the lower part of figure 4, from which it can be seen that in spite of the meagerness of the data there is an evident inverse relationship with the first power of the viscosity.

The passive sinking of *D.* sp. 1 both in its relation to the linear dimensions of the animal and in its relation to the viscosity of the medium thus clearly obeys the generalized form of Stokes' law. It is probable that the largest individuals, for which the length is 1.79 mm., and the velocity of sinking 0.24 cm. per sec. when the viscosity is 0.0097 poise, must come very close to the upper limit for the validity of the law. In such a case Reynold's Number, N, can hardly be very much less than $\frac{0.179 \times 1 \times 0.24}{0.0097} = 4.43$.

In figure 3 the mean sinking speed of three narcotized specimens of *D. pulex*, falling with closed antennae, are plotted from Eyden's data.[4] A fourth specimen which changed density owing to reproduction during the experiment has been excluded. The open circles represent the mean values, for the midpoint in the size ranges, of two experiments not reported in detail. The whole series of five points falls very close to a straight line of slope 1. According to Allen's law, the velocity varies as the appropriate linear dimension less a quantity ξd, as has been indicated above. In the present experiment if the density difference be taken as 0.02,[5] d, will be given by (8) as 0.028 cm. For a sphere ξ is 0.4, for an irregular body 0.28;[6] a value for a falling *Daphnia* of 0.3 would seem reasonable, implying a correction of 0.08 mm. to the linear dimensions of the animals employed. The effect of such a correction would be to reduce the slope of the line of best fit in figure 3 very slightly below unity. It is, however, practically certain that the ratio of the length of the animal to the unknown diameter of the tube employed by Eyden was at least as great, and probably somewhat greater, in her experiments, than was the maximum ratio in those on *D.* sp. 1. The effect of the

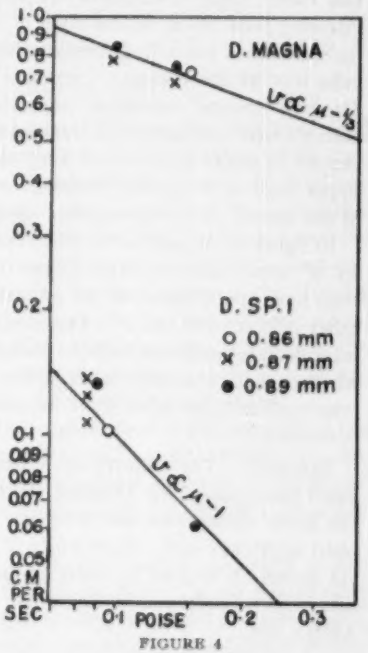

FIGURE 4

Relation of the sinking velocity of *Daphnia magna* (from the data of Bowkiewicz[3]) and of *D.* sp. 1, to viscosity. The crosses, open circles and solid circles indicate different individuals.

Ladenburg correction on Eyden's data would be to increase the slope of the line of best fit. The most reasonable interpretation of figure 3, therefore, is that these two small errors balance each other and that Allen's law holds, at least approximately.

The upper part of figure 4 indicates the relationship between mean sinking velocity and viscosity in experiments by Bowkiewicz with three specimens of *D. magna*, about 2 mm. long. These animals were narcotized, but unlike Eyden's, had open antennae. They certainly were considerably denser than the *D. pulex* employed in her experiments. Bowkiewicz concluded that the sinking speed in this case varied inversely as the square root of the viscosity. He therefore supposed that Newton's law held in this case. Both conclusions are erroneous. Newton's law contains no viscosity term while examination of the figure clearly shows that at least for narcotized animals the sinking speed is inversely proportional to the cube root of the viscosity. Some of Bowkiewicz' experiments with fixed *Daphnia* diverge somewhat from this relationship but so far as living animals are concerned the relationship of the velocity to viscosity is exactly as would be expected from Allen's Law. In these experiments the upper limit of Reynolds' Number, calculated as before, using the length of the animal as the appropriate linear dimension, lies between 10 and 16.

In figure 2 we have given observations on a few specimens of *Daphnia* sp. 2[7] from Bantam Lake, Conn. These animals are somewhat larger than most specimens of *D.* sp. 1 though the increment is mainly due to the taller helmet of *D.* sp. 2. The available evidence seems to suggest some intermediate condition between Stokes' and Allen's laws but it is uncertain whether there is a more or less abrupt transition between the two possible relationships or whether some intermediate value of q, between 1 and 1.5, is implied.

Summary.—The velocity of passive falling of narcotized specimens of small limnoplanktonic *Daphnia* (*D.* sp. 1) is proportional to the square of the linear dimensions and inversely proportional to the viscosity, as implied by Stokes' law; the velocity of passive falling of large pond *Daphnia* (*D. pulex*, *D. magna*) is nearly proportional to the linear dimensions and inversely proportional to the cube root of the viscosity, as implied by Allen's law.

[1] Allen, H. S., "The Motion of a Sphere in a Viscous Fluid," *Phil. Mag. London, Edinburgh, Dublin*, ser. v, **50**, 323–338, 519–534 (1900).

[2] Bowkiewicz, J., "Schwebephase in der Bewegung der Cladoceren und Viskosität des Wassers," *Int. Rev. ges. Hydrobiol. Hydrogr.*, **22**, 146–156 (1929).

[3] *Daphnia* sp. 1 would be called *D. longispina* var. *hyalina* Leydig 1860 form *mendotae* according to Birge (in Ward and Whipple's *Fresh Water Biology*), 1918. A redescription of this species is in preparation by one of us (J. L. B.).

[4] Eyden, D., "Specific Gravity as a Factor in the Vertical Distribution of Plankton,"

Proc. Cambridge Phil. Soc. Biol. Sci. (later *Biol. Rev. Cambridge Phil. Soc.*), **1**, 49–55 (1923).

[5] Lowndes, A. G., "The Displacement Method of Weighing Living Aquatic Organisms," *J. Mar. Biol. Assoc. U. K. Plymouth*, **23**, 555–574 (1942).

[6] DallaValle, J. M., *Micromeritics. The Technology of Fine Particles*, Pitman Publishing Co., New York and London, (1948), XXVIII + 555 pp.

[7] This species has not been described. Woltereck, *Trans. Wisc. Acad. Sci. Madison*, **27**, 487–522 (1932) gives an outline drawing of a *Daphnia* undoubtedly referable to this species as figure 26 (Plate XVI). He has named it *D. longispina apicata* form *nasuta* without any description. A description is in preparation by J. L. B.

NATIONAL ACADEMY OF SCIENCES: MINUTES OF THE MEETING FOR ORGANIZATION, APRIL, 1863

By Edwin B. Wilson

Communicated March 13, 1950

The Report of the National Academy of Sciences for the Year 1863 published in Washington by the Government Printing Office, 1864, contains only the briefest reference to the organization meeting of the Academy It has seemed to the Home Secretary and the Chairman of the Committee on Revision of Constitution that the members of the Academy who have recently been engaged upon a reorganization of the Constitution and Bylaws might be interested in having for reference the Minutes of the Meeting for Organization and a copy of the initial Constitution and Bylaws as considered at that meeting in Committee of the Whole and finally passed at the stated meeting on the 6th of January, 1864. The address of the Hon. Henry Wilson to which reference is made in the Minutes is also appended.

In presenting this material of historic significance to the members of the Academy and to scientists at large, it may be well to call attention to a few remarks of President A. D. Bache in the first annual report of the Academy as revealing the spirit in which the original members and officers approached their tasks.

"The want of an institution by which the scientific strength of the country may be brought, from time to time, to the aid of the government in guiding action by the knowledge of scientific principles and experiments, has long been felt by the patriotic scientific men of the United States. No government of Europe has been willing to dispense with a body, under some name, capable of rendering such aid to the government, and in turn of illustrating the country by scientific discovery and by literary culture.

"It is a remarkable fact in our annals that, just in the midst of difficulties which would have overwhelmed less resolute men, the 37th Congress of the United States, with an elevated policy worthy of the great nation which

they represented, took occasion to bring the scientific men around them in council on scientific matters, by creating the National Academy of Sciences. Such has been the way in which the public mind has been stirred before in the annals of other countries, leading to the organization of government systems of education, science, art, and literature, to be encouraged and perfected when more peaceful and prosperous times recurred.

. . . . . .

"It will be seen, by the spirit and the words of our laws enacted by the authority by the charter, that members of the National Academy put their time and talents at the disposal of the country in no small or stinted measure, freely, fully, by the binding authority of an oath, asking no compensation therefor but the consciousness of contributing to judicious action by the government on matters of science. The more the wealth of such men can be drawn out from the treasury of their knowledge the richer the nation will be; and I, for one, do not fear that even the suggestions which may be made to Congress, of subjects in which that knowledge may be most profitably employed for our country and times, will be subject to any supposed taint of self-seeking as to power or influence. Subject to the taint of supposed desire for remuneration it cannot be, as our charter and all our laws look away from such a centre."

Minutes of the Proceedings
of
The National Academy of Sciences
at the
Meeting held for Organization
in the
Chapel of the New York University
on the
22d, 23d, & 24th days
of
April 1863

In accordance with an appointment made by the Hon. Henry Wilson of Mass. of which due notice had been given, the members of The National Academy of Sciences met in the Chapel of the New York University at 11 a. m. on Wednesday, April 22nd, 1863.

Mr. Wilson was present and called the meeting to order; and after a brief statement of the origin and history of the Bill incorporating the Academy, which was by him introduced into the Senate of the United States called upon Prof. Agassiz, the first named in the Bill, to take the chair. Prof. Agassiz on account of temporary ill health declined the honor tendered him; and after some few pertinent remarks upon the importance of the establishment of the Academy to the progress of Science, and to the general interests of the country, nominated Prof. Joseph Henry of Washington, as chairman, & Prof. Alexis Caswell of R. I. as secretary, pro tempore, both of whom were appointed and entered upon their respective duties.

The following Resolutions offered by Dr. Gibbs of New York were unanimously adopted:

Resolved, that the Academy accepts the Act of Incorporation, & hereby declares its intention of entering with earnestness & devotion upon the high course marked out for it by Congress.

Resolved, that the thanks of the Academy be presented to the Hon. Henry Wilson for the statesmanlike and successful action in the Senate of the United States for the establishment of a National Academy of Sciences; & that he be invited at this and at all other times when agreeable to him, to be present at, and assured of a cordial welcome at, the meetings of the Academy.

The Bill of Incorporation was then read. Thirty-two (32) corporate members were present and answered to their names as follows.

Agassiz, L.	Frazer, J. F.	Alexander, S.	Rogers, F.
Bache, A. D.	Gibbs, W.	Barnard, F. A. P.	Rogers, R. E.
Barnard, J. G.	Gilliss, J. M.	Hubbard, J. S.	Rogers, W. B.
Bartlett, W. H. C.	Gould, B. A.	Leidy, J.	Rutherfurd, L. M.
Caswell, A.	Guyot, A.	Lesley, J. P.	Saxton, J.
Coffin, J. H. C.	Hall, J.	Newberry, J. S.	Silliman, B., Jr.
Dana, J. D.	Henry, J.	Newton, H. A.	Strong, T.
Davis, C. H.	Hilgard, J. E.	Peirce, B.	Winlock, J.

On motion of Prof. B. Peirce it was *Voted* that a committee of nine be appointed by the chair to draft & report a plan of organization. The chair appointed the following: A. Caswell, A. D. Bache, W. B. Rogers, W. Gibbs, J. F. Frazer, B. Silliman, Jr., B. A. Gould, B. Peirce and L. Agassiz.

Opportunity was given for a general interchange of views upon the plan most proper to be adopted.

On motion of Dr. Gould it was *Voted* that members having any propositions to submit to the committee be requested to present them in writing.

On motion of Dr. Gibbs it was *Voted* that the chairman & secretary furnish for the Press such Report of the Proceedings as they may think proper.

On motion of Mr. Hilgard it was *Voted* that the meeting for Organization be with closed doors.

On motion of Mr. Hilgard, it was *Voted* that a committee of five members be appointed by the chairman to prepare & report upon (1) The form of a Diploma, (2) The Corporate Seal, (3) A stamp for Books and Property. Messrs. F. A. P. Barnard, J. E. Hilgard, J. Saxton, L. M. Rutherfurd and J. P. Lesley were appointed.

It was voted that when we adjourn it be to meet at 8 o'clock this evening.

Adjourned.

ALEXIS CASWELL
Sec'y. pro tem.

8 o'clock P. M. April 22nd, 1863.

The chairman called the meeting to order agreeably to adjournment.

The Roll was called. Shortly after Roll Call all the members present at the morning were believed to be present with the exception of James D. Dana, who had left N. Y. for New Haven.

The committee on organization reported through their chairman a series of Articles forty-five (45) in number which were recommended for adoption as the Laws of the Academy.

Prof. S. Alexander moved that the Report be printed and referred to a subsequent meeting for consideration. —Motion lost.

On motion of Dr. B. A. Gould it was *Voted* that the Report be now taken up for consideration Article by Article in order.

Articles 1st, 2d, 3d, 4th, 5th, & 6th were adopted without material alteration.

Dr. Leidy moved that article 7th fixing the form of an oath of Allegiance to be taken by the members be amended by striking out the first part which refers to having borne arms against the Government or in any manner aided persons acting in hostility thereto.

After a somewhat protracted debate the motion was put and lost.

The article was then adopted as reported by the committee.

Articles 8th & 9th were adopted.

On motion of Prof. Frazer Article 10th referring to Elections was amended by substituting the word "*majority*" for the word "*plurality*."

Pending the consideration of the 10th Article the Academy adjourned to meet at 10 a. m. tomorrow.

ALEXIS CASWELL
Sec'y. pro tem.

Thursday April 23d, 10 A. M.

Pursuant to adjournment the meeting was called to order by the chairman.

The Roll was called and the following members answered to their names.

Agassiz	Hubbard	Guyot	Peirce	
Bache	Leidy	Rogers, W. B.	Rogers, F.	
Barnard, F. A. P.	Lesley	Saxton	Rogers, R. E.	
Bartlett	Frazer	Winlock		
Caswell	Gibbs	Torrey	Coffin	came in
Hall	Gilliss	Newberry	Hilgard	after
Henry	Gould, B. A.	Newton	Strong	Roll Call

Prof. Caswell moved a reconsideration of Article 7, fixing the form of the Oath of Allegiance with a view to offer an amendment which would limit its administration in its present form to the duration of the present Rebellion & thus obviate the main objection which had been urged against it.

After a brief debate the motion was withdrawn.

Prof. Peirce gave notice that he would at a later stage of the business offer a substitute for Article 7th.

Article 10th of the organic Laws which was under consideration at the time of adjournment last evening was resumed.

On motion of Prof. Frazer it was amended by substituting the word "*majority*" for the word "*plurality*" in the election of chairman of the classes.

On motion of Prof. Peirce the paragraph on the division of the members into sections was amended by adding the words following, viz., "By a special vote of the Academy a member may inscribe his name in a section of the class to which he does not belong."

Article 10th as amended was then adopted.

On motion of Prof. Bache the committee on Organization was reappointed and Article 11th was referred to them for revision.

Articles 12 to 23 inclusive were adopted.

On motion of Dr. Gould Article 24th was referred to the committee on Organization for revision.

Article 25th was adopted.

On motion of Prof. W. B. Rogers Article 26th fixing the requirement of memoirs and papers from the members was striken out.

On motion of Dr. Gould it was *Voted* that the Academy adjourn at $3^1/_2$ p. m. to meet at 10 a. m. tomorrow.

The remaining articles numbered 27 to 45 inclusive (or in the printed copy 26 to 44) were adopted.

On motion of Prof. Bache, Prof. Winlock was added to the committee on organization.

At 4 p. m. the Academy adjourned to allow a short time for the committee on Organization to revise Articles 11 & 24.

At 4½ p. m. the meeting was again called to order by the chairman.

The committee reported articles 11 & 24 revised: and they were then adopted.

Prof. Peirce agreeably to previous notice moved a substitute for Article 7th (prescribing the form of the oath) in the words following viz., "All ordinary members of the Academy shall be citizens of the United States. Every member shall take the oath of Allegiance prescribed by the Senate of the United States for its own members; and in addition thereto, shall take an oath faithfully to discharge the duties of a member of the National Academy of Sciences to the best of his ability."

After a brief debate the substitute was adopted.

On motion of Dr. Gould Article 16th fixing the time of holding the stated meetings of the Academy was so amended that one of them shall be held on the *third day* of January (or if that be Sunday, on the Monday next following); and the other on the third Wednesday in August.

On motion of Prof. Bache the articles separately passed upon were provisionally adopted as a whole; and a committee of three was appointed to put them immediately in print with a view to a further revision on another day.

Committee—Messrs. Gibbs, Hilgard & Rutherfurd.

On motion of Prof. Frazer it was *Voted* that a committee of three be appointed by the chair to revise the style and arrangement of the articles; and report at the next stated meeting of the Academy.

Adjourned,
ALEXIS CASWELL
Sec'y. pro tem.

Friday 10 a. m. April 24th

Pursuant to adjournment the Academy was called to order by the chairman.

The minutes of the preceding meetings were read & corrected.

On the suggestion of the chairman Mr. F. Rogers was appointed assistant secretary pro tem.

On motion of Prof. Peirce it was *Voted* that in the minutes of the proceedings all titles of members shall be omitted, and the prefix "*Mr.*" used.

The committee on the Revision of the Laws was announced from the chair; viz., Mr. Frazer, Mr. Davis & Mr. Caswell.

On motion of Mr. Rutherfurd it was *Voted* that all the Articles of the Laws with the exception of Article 43th, relating the "Alteration of Laws" (on page 13 of the printed copy) be permanently adopted.

On motion of Mr. Frazer it was *Voted* that the Secretary be directed to call the Roll; and that each member be requested when his name is called to assign the *Class* and *Section* in which he wishes his name to be enrolled.

The roll was called, and the selections were as follows, viz.:

CLASS *A*. MATHEMATICS & PHYSICS.

Sec. 1. *Mathematics*. J. G. Barnard, Peirce, Strong and Winlock.
Sec. 2. *Physics*. Bache, Bartlett, F. A. P. Barnard, Henry and W. B. Rogers.
Sec. 3. *Astronomy, Geography* and *Geodesy*. Caswell, Coffin, Davis, Gilliss, Gould, Hubbard and Rutherfurd.

Sec. 4. *Mechanics*. Frazer, Hilgard, F. Rogers and Saxton.
Sec. 5. *Chemistry*. Gibbs, Silliman, B., Jr., and Torrey.

CLASS *B*. NATURAL HISTORY

Sec. 1. *Mineralogy* and *Geology*. Lesley and Newberry.
Sec. 2. *Zoology*. Agassiz.
Sec. 3. *Botany*.
Sec. 4. *Anatomy* and *Physiology*.
Sec. 5. *Ethnology*.

On motion of Mr. Gibbs it was *Voted* that a committee of two be appointed to arrange a book for the signatures of the members. Mr. Gibbs and Mr. W. B. Rogers were appointed said committee.

The Secretary then administered to the chairman the oath of allegiance to the Government, and of Fidelity to the Academy in the following words. (nomine mutate)

"I (A.B.) do solemnly affirm that I have never voluntarily borne arms against the United States since I have been a citizen thereof; that I have voluntarily given no aid, countenance, counsel or encouragement to persons engaged in armed hostility thereto; that I have neither sought nor expected to exercise the functions of any office whatever under any authority or pretended authority in hostility to the United States; that I have not yielded a voluntary support to any pretended government, authority, power or constitution within the United States, hostile or inimical thereto. And I do further affirm that to the best of my knowledge and ability, I will support and defend the Constitution of the United States against all enemies, foreign and domestic: that I will bear true faith and allegiance to the same; that I take this obligation freely, without any mental reservation, or purpose of evasion; and that I will well and faithfully discharge the duties of a member of the National Academy of Sciences. So help me God."

The chairman then administered the same oath to all the members, whose names are as follows.

Agassiz	Bache	Barnard, F. A. P.	Barnard, J. G.
Bartlett	Caswell	Coffin	Davis
Frazer	Gibbs	Gilliss	Gould, B. A.
Hilgard	Hubbard	Lesley	Newberry
Peirce	Rogers, F.	Rogers, W. B.	Rutherfurd
Saxton	Silliman, B., Jr.	Strong	Torrey
Winlock			

On motion of Mr. Frazer it was *Voted* that the Academy do now proceed to the election of officers.

On motion of Mr. Gibbs it was *Voted* that a committee of two be appointed to collect and count the votes. Messrs. Gibbs and F. A. P. Barnard were appointed the committee.

The chairman after a few remarks of warning and encouragement to the members before leaving the chair called for nominations for President of the Academy.

A. D. Bache was nominated.

The ballot was taken and on the Report of the tellers, *Alexander Dallas Bache* was declared by the chairman to be elected President of the Academy.

The chairman pro tem. then retired from the chair.

Mr. Caswell moved that Mr. Strong be appointed a committee to conduct the President elect to the chair. —Carried.

The President on taking the chair returned his thanks to the Academy for the honor they had done him, and proceeded with the business.

Nominations for Vice-President were called for.

James D. Dana was nominated.

The ballot was taken and *James D. Dana* was declared to be elected Vice-President.

For Foreign Secretary, Louis Agassiz was nominated. The ballot was taken and *Louis Agassiz* was declared to be elected.

For Home Secretary, Wolcott Gibbs was nominated. The ballot was taken and *Wolcott Gibbs* was declared to be elected. The Home Secretary requested the secretary pro tem. to retain his place till the close of the meeting.

For Treasurer, Fairman Rogers and L. M. Rutherfurd were nominated. The ballot was taken and *Fairman Rogers* was declared to be elected.

On proceeding to the election of Councillors, doubts were expressed whether it could with propriety be done at this time inasmuch as it was uncertain whether members not present would accept their appointment as Academicians under the Bill. Whereupon statements of members present showed that the following persons named in the Bill of Incorporation, but not present at this meeting, had signified their intention to accept their appointments as Academicians; viz., Mr. B. Silliman, Sen., Mr. J. Wyman, Mr. A. Gray, Mr. J. L. Leconte, Mr. G. Engelmann, Mr. W. Chauvenet, Mr. M. F. Longstreth and Mr. John Rodgers.

On motion of Mr. Frazer it was *Voted* that we proceed to the election of four members of the council.

Nominations for councillors were then called for, when the following names were announced.

Mr. Henry, Mr. Frazer, Mr. W. B. Rogers, Mr. Davis, Mr. Rutherfurd, Mr. Torrey, Mr. Lesley, Mr. Gilliss, Mr. Newberry and Mr. Gray.

Mr. Henry expressed a wish not to be elected, and hoped he might be allowed to withdraw his name.

After the first ballot, on Report of the tellers, Mr. *Charles H. Davis* was declared to be elected.

After the second ballot, Mr. *John Torrey* was declared to be elected.

After the third ballot, Mr. *L. M. Rutherfurd* was declared to be elected.

After the fourth ballot, Mr. *J. P. Lesley* was declared to be elected.

On motion of Mr. Frazer the committee on elections was discharged.

On motion of Mr. Davis it was *Voted* that the address with which the Hon. Henry Wilson inaugurated the first meeting of the National Academy of Sciences be entered upon the Journal in full; and that he be requested to furnish a copy for that purpose.

On motion of Mr. Frazer it was *Voted* that the thanks of the Academy be returned to the temporary chairman and secretary for the able manner in which they have discharged their duties.

Mr. B. Silliman, Jr., moved a recess for half an hour, or from 1:45 to 2:15 p. m. Carried; and the meeting adjourned.

At 2:15 the meeting was called to order by the President.

On motion of Mr. B. Silliman, Jr., a further recess was voted for a short time in order to give Class *A* an opportunity to organize.

At 2:45 p. m. the meeting was again called to order by the President.

Reports of the organization of classes were received, from which it appeared that the following class officers had been elected. Viz.—

Class A. Benjamin Peirce, chairman.
Benjamin A. Gould, secretary.

Class B. Benjamin Silliman, Sen., chairman.
J. S. Newberry, secretary.

The committee on the Diploma and Seal reported progress and also the following resolution which was passed, viz.,

Resolved that the committee on the Diploma and Seal be continued with instructions to report at the next stated meeting; and that Mr. F. Rogers and Mr. C. H. Davis be added to the committee.

On motion of Mr. Frazer it was *Voted* that in article 18 in the printed Laws (on pp. 6 & 7) another specification be added in the following words "12 rough minutes read for correction." And also that in No. 3 of the same article, the word "*correction*" be striken out and the word "*adoption*" inserted in its place.

On motion of Mr. Frazer it was *Voted* that the August stated meeting of this year be dispensed with.

On motion of Mr. Gibbs it was *Voted* that the thanks of the Academy be tendered to Chancellor Ferris for the use of Rooms in the University.

Mr. F. Rogers moved to amend Article 27th of the Printed Laws on p. 9 by adding at the beginning of the second paragraph the words "*short communications or*," and by substituting for the word "*printed*" the words, "*published without delay*." Carried.

On motion of Mr. Frazer article 27 of the printed laws on p. 9 was amended by adding at the close of it the words following, viz., "*The Academy will not hold itself responsible for the opinions expressed in such papers.*"

Mr. B. A. Gould moved the following resolutions.

Resolved that no more than ten Foreign Associates be elected at any one stated meeting. Carried.

On motion of Mr. B. A. Gould article 5th on page 2 of the printed Laws was amended by inserting at the beginning of the second paragraph the words, "*For ordinary members.*"

Mr. Gould also moved that a committee of three be appointed to draft and present to the committee on Revision for incorporation in their Report. *A Rule prescribing the mode of electing Foreign Associates.*—Carried. Mr. Agassiz, Mr. Gould, and Mr. B. Silliman, Jr., were appointed said committee.

On motion of Mr. Frazer it was *Voted* that the President be requested to place his name on the committee of weights and measures when appointed.

At 4 o'clock p. m. on motion of Mr. Peirce, The Academy adjourned to meet in the city of Washington on the third day of January 1864.

ALEXIS CASWELL
Sec'y. pro tem.

(From REPORT OF THE NATIONAL ACADEMY OF SCIENCES for 1863, Washington, 1864, pp. 113–118).

Constitution and By-laws of the National Academy of Sciences

PREAMBLE

Empowered by the act of incorporation, adopted by Congress, and approved by the President of the United States on the 4th day of March, A.D. 1863, the National Academy of Sciences do enact the following constitution and by-laws:

ARTICLE I.

Of members.

SECTION 1. The members of the Academy shall be designated as members, honorary members, and foreign associates.

SEC. 2. The Academy shall consist of the fifty members named in the act of incorporation, and of such others, citizens of the United States, as shall from time to time be elected to fill vacancies, in the manner hereinafter provided.

SEC. 3. Every member shall, upon his admission, take the oath of allegiance prescribed by the Senate of the United States for its own members, and, in addition thereto, an oath faithfully to discharge the duties of a member of the National Academy of Sciences to the best of his ability. He shall also subscribe the laws of the Academy.

SEC. 4. The members of the Academy shall be arranged in two classes, according to their special studies, viz.: A, the class of mathematics and physics, and B, the class of natural history. The corporate members may select the class in which they desire to be arranged.

SEC. 5. The members of the classes shall arrange themselves in sections by inscribing their names under one of the following heads: Class A, *mathematics and physics;* sections—1, mathematics; 2, physics; 3, astronomy, geography, and geodesy; 4, mechanics; 5, chemistry.

Class B, *natural history;* sections—1, mineralogy and geology; 2, zoology; 3, botany; 4, anatomy and physiology; 5, ethnology.

But the Academy retains the power of transferring a member from one section to another.

SEC. 6. A member may be elected an honorary member of any section by a vote of a majority of such section.

SEC. 7. The Academy may elect fifty foreign associates, who shall have the privilege of attending the meetings of the Academy and of reading and communicating papers to it, but shall take no part in its business, and shall not be subject to its assessments.

They shall be entitled to a copy of the publications of the Academy.

ARTICLE II.

Of the officers.

SEC. 1. The officers of the Academy shall be a president, a vice-president, a foreign secretary, a home secretary, and a treasurer; all of whom shall be elected for a term of six years by a majority of votes present at the first stated session after the expiration of the current terms, provided that existing officers retain their places until their successors are elected. In case of a vacancy, the election for six years shall be held in the same manner at the next stated session after the vacancy occurs.

SEC. 2. The officers of the classes shall be a chairman and a secretary, who shall be elected at each January session. The nominations shall be open, and a majority of votes shall be necessary to elect.

SEC. 3. The officers of the Academy and the chairmen of the classes, together with four members, two from each class, to be annually elected by the Academy, at the January session, by a plurality of the votes, shall constitute a council for the transaction of such business as may be assigned to them by the constitution or the Academy.

SEC. 4. The president of the Academy, or in case of his absence or inability to act, the vice-president, shall preside at the meetings of the Academy and of the council; shall name all committees, except such as are otherwise especially provided for; refer investigations required by the government of the United States to members specially conversant with the subject, and report thereon to the Academy at its next January session, and, with the council, shall direct the general business of the Academy.

It shall be competent for the president in special cases to call in the aid upon committees of experts or men of remarkable attainments, not members of the Academy.

SEC. 5. The foreign and home secretaries shall conduct the correspondence proper to their respective departments, advising with the president and council in cases of

doubt, and reporting their action to the Academy at its January session. It shall be the duty of the home secretary to give notice to the members of the place and time of all meetings, and to make known to the council all vacancies in the list of members.

The minutes of each session shall be duly engrossed before the next stated session, under the direction of the home secretary.

SEC. 6. The treasurer shall attend to all receipts and disbursements of the Academy, giving such bond and furnishing such vouchers as the council may require. He shall collect all dues from members, and keep a set of books showing a full account of receipts and disbursements. He shall present at each stated session a list of the members entitled to vote, and a general report at the January session. He shall be the custodian of the corporate seal of the Academy.

ARTICLE III.

Of the meetings.

SEC. 1. The Academy shall hold two stated sessions in each year: one in the city of Washington, on the third day of January (unless that day falls on Sunday, when the session shall be held on the succeeding Monday); and one in August, at such time and place as the Academy shall have determined upon, in private meeting, on the last day of the preceding January session.

SEC. 2. The names of the members present at each daily meeting shall be recorded in the minutes; and the members present at any meeting shall constitute a quorum for the transaction of business.

SEC. 3. Scientific meetings of the Academy, unless otherwise ordered by a majority of the members present, shall be open to the public; those for the transaction of business closed.

SEC. 4. The Academy may divide into classes for scientific or other business. In like manner, the classes may divide into sections.

SEC. 5. The classes shall meet during such periods of the stated meetings of the Academy as may be fixed by the Academy. Special meetings of a class may be called by the council at the request of five members of the class.

SEC. 6. The stated meetings of the council shall be held at the times of the stated or special meetings of the Academy. Special meetings shall be convened at the call of the president and two members of the council, or of four members of the council.

SEC. 7. No member who has not paid his dues shall take part in the business of the Academy.

ARTICLE IV.

Of elections, regulations, and expulsions.

SEC. 1. All elections shall be by ballot, unless otherwise ordered by this constitution; and each election shall be held separately.

SEC. 2. Whenever any election is to be held, the presiding officer shall name a committee to conduct it, to collect the votes, count them, and report the result to the Academy. The same law shall apply in the classes.

SEC. 3. Nominations for officers shall be made at the close of the first daily meeting of a stated session; and no candidate shall be voted for unless thus nominated.

SEC. 4. For election of members the council shall first decide the class in which the vacancy shall be filled. Each section of that class may then select one or more candidates after a discussion of their qualifications, and present their claims to the class, who shall select three to be presented in the order of their preference to the Academy; from these three the Academy shall elect by a majority of the members present. The member elect shall be assigned to the section in which he has been proposed. The Academy may nominate candidates in any section which fails to propose them for itself.

SEC. 5. Every member elect shall accept his membership personally or in writing before the close of the next stated session after the date of his election. Otherwise, on proof that the secretary has formally notified him of his election, his name shall not be entered on the roll of members.

SEC. 6. Elections of foreign associates shall be conducted as follows:

Each section shall report to its class, nominating a candidate whose special researches need not belong within the province of the section, but must be comprised within the range of the class.

From these candidates each class shall select one name to be presented to the Academy, and from these two names the Academy, after full discussion, shall make the election, at such time as it may have previously appointed for the purpose.

SEC. 7. A diploma, with the corporate seal of the Academy and the signatures of the officers, shall be sent by the appropriate secretary to each member on his acceptance of his membership.

SEC. 8. Resignations shall be addressed to the president and acted on by the Academy. No resignation of membership shall be accepted unless all dues have been paid.

SEC. 9. Members resigning in good standing will retain an honorary membership; being admitted to the meetings of the Academy, but without taking part in the business. Honorary members will not be liable to assessment.

SEC. 10. If any member be absent from four consecutive stated meetings of the Academy without communicating to the Academy a valid reason for his absence, his name shall be striken from the roll of members.

SEC. 11. Members and officers habitually neglecting their duties shall be impeached by the council, and at once notified thereof in writing by the home secretary.

SEC. 12. Impeachments of members or officers shall first be tried before the council, which may be convened specially for such purpose. If it decides that the impeachment is proper, such impeachment shall be tried in private session before the Academy at its next stated meeting.

SEC. 13. The expulsion of a member shall be formally and publicly announced by the president at the stated session during which expulsion shall take place.

ARTICLE V.

Of scientific communications, publications, and reports.

SEC. 1. Papers on scientific subjects may be read at the meetings of the Academy, or of the classes or sections to which the subject belongs.

SEC. 2. Any member of the Academy may read a paper from a person who is not a member, and shall not be considered responsible for the facts or opinions expressed by the author, but shall be held responsible for the propriety of the paper.

SEC. 3. The Academy shall provide for the publication, under the direction of the council, of proceedings, memoirs, and reports.

SEC. 4. Propositions for investigations or reports shall originate with the classes to which the subjects belong, and be by them submitted to the Academy for approval, except requests from the government of the United States, which shall be acted on by the president, who will in such cases report, if necessary, at once to the government, and to the Academy at its next stated meeting.

SEC. 5. The judgment of the Academy shall be at all times at the disposition of the government upon any matter of science or art within the limits of the subjects embraced by it.

SEC. 6. An annual report, to be presented to Congress, shall be prepared by the president, and before its presentation submitted by him first to the council, and afterwards to the Academy at its January meeting.

SEC. 7. Medals and prizes may be established, and the means of bestowing them accepted by the Academy upon the recommendation of the council, by whom all the necessary arrangements for their establishment and award shall be made.

ARTICLE VI.

Of the property of the Academy.

SEC. 1. All investments shall be made by the treasurer in the corporate name of the Academy in stocks of the United States.

SEC. 2. No contract shall be binding upon the Academy which has not been first approved by the council.

SEC. 3. The assessments required for the support of the Academy shall be fixed by the Academy on the recommendation of the council.

ARTICLE VII.

Of additions and amendments

Additions and amendments to the constitution shall be made only at a stated session of the Academy. Notice of a proposition for such a change may be given at any stated session, and shall be referred to the council, which may amend the proposition, and shall report thereon to the Academy at its next stated session, with a recommendation that it be accepted or rejected. Its report shall be considered by the Academy in committee of the whole, and immediately thereafter acted on. If the addition or amendment receive two-thirds of the votes present, it shall be declared adopted, and shall have the same force as the original law.

BY-LAWS.

Of the officers.

I. In the absence of the chairman or secretary of a class, a member shall be chosen to perform his duties temporarily, by a plurality of the *viva voce* votes, upon open nomination.

II. The accounts of the treasurer shall be referred to an auditing committee of three members, to be appointed by the Academy at the meeting at which the accounts are presented; which committee shall report before the close of that session, and shall then be discharged.

Of the meetings

III. A committee of arrangements, for each stated session of the Academy, of five members, shall be appointed by the president, the class secretaries to be *ex officio* two of the members of the committee. This committee shall meet not less than two weeks previous to each meeting. It shall be in session during the meetings to make arrangements for the reception of the members; to arrange the business of each day; to receive the titles of papers, reports, &c.; and to arrange the order of reading, and in general to attend to all business and scientific arrangements.

IV. At the meetings the order of business shall be as follows:

1. Chair taken by the president, or, in his absence, the vice-president.
2. Roll of members called by home secretary.
3. Report by treasurer of members entitled to vote.
4. Minutes of the preceding meeting read and approved.
5. Stated business.

6. Reports of president, secretaries, treasurer, classes, and committees.
7. Business from council.
8. Other business.
9. Communications from members.
10. Communications from persons not members.
11. Announcements of the death of members. Biographical notices read.
12. Rough minutes read for correction.

V. The rules of order of the Academy shall be those of the Senate of the United States, unless otherwise directed.

VI. It shall be in order for twelve members to require that any matter of business be discussed in committee of the whole for amendment; the vote upon amendments to be taken in the whole Academy; and the amended proposition or propositions to be similarly voted on.

VII. The scientific meetings shall be convened at twelve o'clock m., in order to allow time for the business meetings of the Academy, and for the meetings of classes, sections, and committees.

Of elections and obituaries.

VIII. No more than ten foreign associates shall be elected at any one stated session.

IX. The death of members shall be announced by the president on the last day of each stated session, when a member shall be selected by the Academy to furnish a biographical notice of the deceased at the next stated session. If such notice be not then furnished, another member shall be selected by the Academy in place of the first, and so on until the duty is performed.

X. The deaths of such eminent scientific men of the country as have taken place since the last session of the Academy shall be announced by the president. The names shall be selected by the council.

Of scientific communications, publications, and reports.

XI. An analysis of the memoirs and reports read in the meeting of the classes shall be given by the secretaries of the classes to the home secretary for publication in the proceedings of the Academy. For any failure in this duty, the delinquent officer shall be impeached by the home secretary.

XII. The secretaries shall receive memoirs at any time, and report the date of their reception at the next session. But no memoir shall be published unless it has been read before the Academy, class, or section, and ordered to be published by the Academy. Papers shall be published in the order in which they were registered, but papers which have not been sent to the secretary within a month from the time of their reading shall not be published without a special vote of the Academy.

XIII. Memoirs shall date in the records of the Academy from the day of their presentation to the Academy, and this order of their presentation shall be that in which they were registered, unless changed by consent of the author.

XIV. The publication of any communication to which remonstrance is made by the section to which the subject belongs shall be suspended until a second time authorized by a vote of the Academy.

XV. Papers from persons not members, read before the Academy, classes, or sections, and intended for publication, shall be referred, at the meeting at which they are read, to a committee of members competent to judge whether the paper is worthy of publication. Such committees shall report to the Academy as early as practicable, and not later than the next stated session. If they do not then report they shall be discharged and the paper referred to another committee.

XVI. Abstracts of papers published in the transactions of other societies or in journals may be communicated orally to the Academy; and if, on submitting any such communication to a committee, its publication be approved, it may be ordered for publication on a vote of the Academy.

XVII. Short communications or abstracts of memoirs may be sent by any member to the home secretary, who shall, if requested by the author, without delay circulate them among the members.

XVIII. An annual of the Academy shall be prepared by the secretaries, and published on the first day of each year.

XIX. The printing of the Academy shall be under the charge of the secretaries and the treasurer, as a committee of publication, who shall report in relation thereto at each, January meeting of the Academy.

XX. The annual report of the Academy may be accompanied by a memorial to Congress, in regard to such investigations and other subjects as may be deemed advisable, recommending appropriations therefor when necessary.

XXI. The home secretary shall present to the council estimates for books and stationery, binding, &c., required for the use of the Academy.

Of the property of the Academy.

XXII. The proper secretary shall acknowledge all donations made to the Academy, and shall report them at the next stated session.

XXIII. The books, apparatus, archives, and other property of the Academy shall be deposited in some safe place in the city of Washington. A list of the articles deposited shall be kept by the home secretary, who is authorized to employ a clerk to take charge of them.

XXIV. A stamp corresponding to the corporate seal of the Academy shall be kept by the secretaries, who shall be responsible for the due marking of all books and other objects to which it is applicable.

Labels or other proper marks of similar device shall be placed upon objects not admitting of the stamp.

Of changes in the by-laws.

XXV. Any by-law of the Academy may be amended or repealed on the written motion of any two members, signed by them, and presented at a stated session of the Academy; provided the same shall be approved by a majority of the members present at the next stated session.

From ANNUAL, NATIONAL ACADEMY OF SCIENCES, 1863–4–5–6

Address of the HON. HENRY WILSON

Delivered at the Opening of the First Session of the Academy, April 22, 1863.

Gentlemen:—I hold in my hand the Act, passed in the closing hours of the Thirty-seventh Congress, "To incorporate the National Academy of Sciences." In compliance with many kind requests I am here to call the corporators to order. In rising to perform this agreeable task, I crave for a moment your indulgence.

This Act, under which you have met to organize, incorporates in America, and for America, a National Institution, whose objects, ranging over the illimitable fields of science, are limited only by the wondrous capacities of the human intellect. Such an institution has been for years in the thought and on the tongue of the devotees of science,

but its attainment seemed far in the future. Now it is an achieved fact. Our country has spoken it into being, in this "dark and troubled night" of its history, and commissioned you, gentlemen, to mould and fashion its organization, to infuse into it that vital and animating spirit that shall win in the boundless domains of science the glittering prizes of achievement that will gleam forever on the brow of the nation.

When, a few months ago, a gentleman, whose name is known and honored in both hemispheres, expressed to me the desire that an Academy of Physical Sciences should be founded in America, and that I would at least make the effort to obtain such an act of incorporation for the scientific men of the United States, I replied, that it seemed more fitting that some statesman of ripe scholarship should take the lead in securing such a measure, but that I felt confident I could prepare, introduce, and carry through Congress a measure so eminently calculated to advance the cause of science, and to reflect honor upon our country. I promptly assumed the responsibility, and with such aid and suggestions as I could obtain, I prepared, introduced, and by personal effort with members of both Houses of Congress, carried through this act of incorporation without even a division in either House.

The suggestion was sometimes made that the nation is engaged in a fearful struggle for existence, and the moment was not well chosen to press such a measure. But I thought otherwise. I thought it just the fitting time to act. I wanted the *savans* of the old world, as they turn their eyes hitherward, to see that amid the fire and blood of the most gigantic civil war in the annals of nations, the statesmen and people of the United States, in the calm confidence of assured power, are fostering the elevating, purifying, and consolidating institutions of religion and benevolence, literature, art and science. I wanted the men of Europe, who profess to see in America the failure of republican institutions, to realize that the people of the United States, while eliminating from their system that ever-disturbing element of discord, bequeathed to them by the colonial and commercial policy of England, are cherishing the institutions that elevate man and ennoble nations. The land resounds with the tread of armies, its bright waters are crimsoned, and its fields reddened with fraternal blood. Patriotism surely demands that we strive to make this now discordant, torn, and bleeding nation one and indivisible. The National Academy of Sciences will, I feel sure, be now and hereafter another element of power to keep in their orbits, around the great central sun of the Union, this constellation of sovereign commonwealths.

This act of incorporation may not be, is not, perfect. The task has been one of difficulty and delicacy. The number of members must be limited, while the most eminent men of science must be recognized, and sectional claims harmonized. If unintentional injustice has been done to any one, if mistakes have been made, time will, I trust, correct the injustice and the mistakes. Changes will surely come. "Death is in the world," and this original list of honored names will not remain long unbroken. If men of merit have been forgotten in this act of incorporation, the Academy should seize the first and every occasion to right the seeming wrong.

This Academy is destined, I trust, to live as long as the republic shall endure, and to bear upon its rolls the names of the *savans* of coming generations. Let it then advance high its standard. Let it be as inflexible as justice, and as uncompromising as truth. Let it speak with the authority of knowledge, that pretension may shrink abashed before it, and merit everywhere turn to it confident of recognition.

In the Providence of God, the Thirty-seventh Congress was summoned to the consideration of measures of transcendent magnitude. It enacted measures, empowering the government to raise hundreds of millions of dollars and millions of men, to protect the menaced life of the nation and preserve the vital spirit of freedom. It dealt with great questions of revenue and of finance. It obliterated an abhorrent system from the na-

tional capital, and engraved freedom upon every rood of the national territory. It consecrated the public domain to homesteads for the homeless and landless, and authorized the construction of a railway to unite the Atlantic and the Pacific seas. The enactment of this act to incorporate the Academy of Sciences was not the least in the long list of acts the Thirty-seventh Congress gave to the country, which will leave their impress upon the nation for ages yet to come. It was my fortune to take a humble part in these great measures of legislation. It is a source of profound gratification to me, that, amid the pressure of public affairs, I have been enabled to contribute something to found this Academy for the advancement of the physical sciences in America. It will ever be among my most cherished recollections, that I have been permitted through your courtesy to unite with you in organizing this National Academy, which, we fondly hope, will gather around it, in the centuries yet to come, the illustrious sons of genius and of learning, whose researches will enrich the sciences, and reflect unfading lustre upon the republic.

ERRATA

In the article "Absolute and Unconditional Convergence in Normed Linear Spaces" by A. Dvoretzky and C. A. Rogers, these PROCEEDINGS, **36**, 192–197 (1950), the following corrections should be made on page 197.

	for	read
Lines 4 and 9 from top:	Lemma 3	Theorem 5A
Formula (12):	n_n	n^n
Line 7 from bottom:	$S \subset K \subset \mathcal{P}$	$S \subset C \subset \mathcal{P}$

INFORMATION TO CONTRIBUTORS

THE PROCEEDINGS is the official organ of the NATIONAL ACADEMY OF SCIENCES and of the NATIONAL RESEARCH COUNCIL for the publication of brief accounts of important current researches of members of the ACADEMY and of the COUNCIL and of other American investigators. The PROCEEDINGS will aim especially to secure prompt publication of original announcements of discoveries and wide circulation of the results of American research among investigators in other countries and in all branches of science.

ARTICLES should be brief. The viewpoint should be comprehensive in giving the relation of the paper to previous publications of the author or of others and in exhibiting, where practicable, the significance of the work for other branches of science. Elaborate technical details of the work and long tables of data should be avoided, but authors should be precise in making clear the new results and should give some record of the methods and data upon which they are based.

MANUSCRIPTS should be prepared with a current number of the PROCEEDINGS as a model in matters of form, and should be typewritten in duplicate with double spacing, the author retaining one copy. Illustrations should be confined to text figures of simple character, though more elaborate illustration may be allowed in special instances to authors willing to pay for their preparation and insertion. Particular attention should be given to arranging tabular matter in a simple and concise manner.

REFERENCES to literature, numbered consecutively, will be placed at the end of the article and short footnotes should be avoided. It is suggested that references to periodicals be furnished in some detail and in general in accordance with the standard adopted for the Subject Catalogue of the International Catalog of Scientific Literature, viz., name of author, with initials following (ordinarily omitting title of paper), abbreviated name of Journal, with place of publication, series (if any), volume, inclusive pages, year. For example: Montgomery, T. H., *J. Morph.*, *Boston*, **22**, 781–815 (1911); or, Wheeler, W. M., *Konigsberg, Schr. physik. Ges.*, **35**, 1–142 (1914).

MANUSCRIPTS may be sent to the Managing Editor, Edwin Bidwell Wilson, Harvard School of Public Health, 695 Huntington Ave., Boston, Mass., only by members of the ACADEMY or by chairmen of the divisions of the NATIONAL RESEARCH COUNCIL, who will assume responsibility for the propriety and scientific standards of the paper. Articles are limited to six printed pages; excess space is billed to the author at $8.00 per page.

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